Systemic upregulation of MTP2- and HMA2-mediated Zn partitioning to the shoot supplements local Zn deficiency responses of Arabidopsis

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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2018-RA-00207 1st Editorial decision – *revision requested* May 22, 2018

On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in The Plant Cell. This acceptance is contingent on revision based on the comments of our reviewers. We think you should be able to address these comments on a point-by-point basis as described in the reviewer comments below.

In the absence of a third review, the Guest Editor highlights an additional point for consideration. The abstract implies that your results are largely confirmatory. It would be good if you could highlight in the abstract where the major advances lie, without, of course, overselling your findings.

---------- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2018-RA-00207R1 1st Revision received July 9, 2018

Reviewer comments and author responses:

RESPONSE TO EDITOR: The abstract text was re-organized and modified.

Reviewer #1:

A better understanding of Zn homeostasis regulation in plants and in particular the regulation of zinc deficiency is relevant and timely, considering, as the authors mention, the global problem of zinc deficiency in agricultural soils and its consequences in crop quality and human nutrition.

The findings in this manuscript, describing the identification of MTP2 and HMA2 as key targets of shoot-to-root communication of Zn status, and having an additive function in enhancing root-to-shoot Zn partitioning under Zn deficiency (line 527-539), are very interesting and add new perspectives to the topic. However, I found that the study, as presented here, was not always clear to follow, and I have the following concerns:

Point 1. The use of "root-to-shoot defect in zinc translocation in the *hma2hma4* mutant as a tool to dissect Zn homeostasis which enables a separation of locally regulated from systemically regulated Zn deficiency responses"

(line 385-7, Fig S1) should be better explained. The genes with congruent changes from the root microarray analysis were considered to indicate systemic regulation (Line 190-9, 212-3). Please provide the arguments for this inference. This inference is pivotal to the way the article is written but the argumentation appears not to have been put forward clearly.

RESPONSE: Thank you for pointing out that we are not making ourselves clear.

Changes made:

We ended the sentence beginning in line 186 earlier, in line 189 ("… medium.")

We modified the following text by adding "Conversely," (instead of "whereas"), then continuing with the previous text, adding at the end of this sentence "more Zn- sufficient than roots of wild-type plants grown in control medium."

We are now beginning a new paragraph after line 189 entitled "The Systemic Zinc Deficiency response".

We inserted in line 194 (after "Data Sets 1 and 2)": "In more detail, we first identified transcripts that differed in abundance between roots of *hma2 hma4* plants and the wild type cultivated under control conditions (see Supplemental Figure 1A). Among these, we expected root transcripts that respond systemically to the deficient Zn status of shoots, as well as transcripts responding to the locally elevated Zn status in roots characteristic of the double mutant (see Supplemental Figure 1B, left and right, and 1D, left circle). To identify the subset of root transcripts which respond systemically to the Zn status of the shoot, we intersected the former list of transcripts with the transcripts that differed in abundance between roots of Zn-deficient and Zn-sufficient wild-type plants (see Supplemental Figure 1B, left and center, and Figure 1D, right circle)."

In line 196, we replaced "congruent" with "co-directional".

Point 2. The information on the microarray analysis should be more clear. The Methods section refers the analysis of root and shoot tissue (Line 673), but the Results section only addresses the root transcriptomic analysis (Line 190-9, Table 1, Fig1D), whereas the Discussion refers to transcripts from the shoot microarray as candidate genes for the involvement in signal generation and propagation (Lines 511-14, Table S1). I miss a clear initial explanation on the microarray analysis, why were root and shoot tissue used, what can be inferred from either and why.

RESPONSE: In line 209 of the results section, we mention the comparison in shoots.

Changes made:

We added an explanation by adding an additional sentence after the end of line 209. "The resulting group of transcripts comprises the local Zn deficiency of shoots, for example *ZIP4*, *ZIP5*, *ZIP9*, *IRT3* and *NAS3*, including candidates that may participate in the generation of a long-distance signal, for example *Phloem Protein 2-B15* (AT1G09155), *Serine CarboxyPeptidase-Like* genes (*SCPL15*, AT3G12240, and *SCPL22*, AT2G24000), and genes encoding kinases, phosphatases and transcription factors."

At the end of the sentence in line 213, we added "in roots".

We ended the sentence in line 215 after media. We changed the next sentence to include more detail, i.e., plant age at harvest and hydroponic cultivation

We re-arranged and expanded the Methods section to clarify how plants were grown for the microarray hybridizations.

Point 3. In line with this, the information in Methods section of the WT -Zn vs control transcriptomic comparison only refers to the reference Talke et al, 2006. In order for readers to be assured that this comparison is valid, information should be provided on the plant material used (e.g. age, growth conditions). Possible differences between the microarrays in this respect (e.g. developmental stage) should be acknowledged and discussed.

RESPONSE: Part of this is explained in the methods (lines 558 to 574 of first submission). Briefly, we fully agree that environmental, age, size or developmental differences are expected to cause differences between the genes identified by the two comparisons made. Here we specifically identify commonalities that are then unaffected by different growth conditions, treatment times or different plant age, i.e. robust commonalities. Consequently, we expect to have false negatives through our approach, e.g., those genes responding to Zn

deficiency in a developmental stage- or environment- specific manner. Importantly, we do not expect to generate false positives in this manner. All plants were in vegetative stage at the time of harvest.

Changes made:

We added more information concerning the hydroponic growth conditions employed for microarray hybridization as described in more detail in the reference Talke *et al*. (2006), continuing the sentence ending in line 560: ", using 6-week-old vegetative plants cultivated in 11 h light (20°C, 145 µmol m^{-2} s^{-1})/13 h dark (18°C), with -Zn (no Zn added to solution) or +Zn (control, 5 µM Zn) conditions for three weeks before harvest. "

Because we could not find another appropriate place to discuss this, we added after line 696: "By choosing those genes commonly regulated in both cultivation systems (see Figure 1D and Supplemental Figure 1A), we identified the subset of co-responding genes irrespective of plant age and environmental conditions."

Point 4. The Zn root state in the *hma2 hma4* mutant at control conditions had become Zn excess (Fig S1A). Thus in the transcriptomic comparison between *hma2 hma4* and WT there are two factors changing, not only the Zn deficient shoot in *hma2 hma4*, instead of Zn sufficient shoot in WT, but also the Zn excess in root instead of Zn sufficient root in WT. Perhaps the authors should address and discuss this. It would be perhaps relevant to verify the *MTP2* gene expression in a WT root transcriptomic comparison between Zn sufficient and Zn excess, or qPCR analyses, to validate the MTP2 systemic regulation.

RESPONSE: The *hma2hma4* mutant is generally a very sick mutant and responded equally positively to Zn supplementation at different concentrations in the normal (non-excess) range, despite a moderate overaccumulation of Zn in roots. It should be noted also that the microarrays were conducted with *hma2hma4* and wild-type plants grown in agar-solidified modified Hoagland's medium containing 1 µM Zn, whereas the data in Figure 1A and S1B were obtained using modified Hoagland's solutions containing a higher Zn concentration of 5 µM. *MTP2* transcript levels do not increase to a biologically relevant degree (less than one C_T on average) under excess Zn conditions in the wild type (30 µM Zn instead of 1 µM Zn in medium of the microarray experiment) (see new Figure S1D; compare with Figure 2A).

Our first submission provided independent evidence for the systemic regulation of *MTP2* in wild-type plants in Fig. 2C and D. By expanding the explanation in response to reviewer #1, point 1, we believe that we responded to this comment, as well.

Note that, to expand on the 3rd listed change made in response to reviewer #1, point 1, we would expect that a transcript which increases in abundance in roots in response to increased root Zn in *hma2hma4* compared to the wild type would NOT increase in abundance in roots of Zn deficient compared to Zn-sufficient wild-type plants. It follows from this that, if the increase in root *MTP2* transcript levels in *hma2 hma4* were a response to local Zn accumulation in roots (and not Zn deficiency in shoots), then we would not have identified this gene as a candidate for systemic regulation in our microarray study.

Changes made:

We added the diagram mentioned above as Figure S1D. We inserted the following text after line 229 of the first submission: "Root *MTP2* transcript levels were more than 2-fold upregulated upon 1 w exposure to elevated concentrations of Co and Cd, but less in response to excess Mn, Fe, Zn, or Mn deficiency (Supplemental Figure 1D."

We inserted a "moderately" when describing the results of Fig. S1B in order not to confuse the reader. Similarly in line 144 of the introduction.

Point 5. I think there are some major discrepancies in the root Zn concentration data. In Fig S1B, 19-d-old WT seedlings have root Zn concentration at control around 300-400 ug g-1 dw, and in Fig S2D, the same 19-d-old WT seedlings have root Zn concentration at control around 40 ug g-1 dw, while in Fig S3F, 7-week-old WT plants have root Zn concentration at control around 40-50 ug g-1 dw. Perhaps some mixing up of data from agar- or hydroponics-grown plants? Please explain or correct these differences.

RESPONSE: Thank you for finding and pointing out these discrepancies. This is indeed surprising. We checked all our ICP data and experiments in detail, and we are very sure not to have mixed up our data. While checking, we did find minor errors in our calculations for Fig. 1A, which are now fixed, but hardly change the results. These changes do not affect the conclusions from this panel.

For Figures 1A and S1B, seeds were germinated on agar-solidified 0.5xMS media (15 µM Zn) for 7 d before transfer to fresh Hoagland's-based treatment media and cultivation for another 14 d. Similarly, for the microarray comparison between double mutant and the wild type, seedlings were transferred to fresh agar-solidified Hoagland's medium after 7 d. Different from Figures 1A and S1B, for Figure S2D, seeds were germinated directly on EDTA-washed Hoagland's-based treatment media and cultivated on these same media for the entire growth period (i.e., never transferred to fresh media). This explains the higher tissue Zn concentrations in these Figures than in Figure S2D. We are very sorry that this difference in experimental design was not evident in the methods of the first submission.

Changes made:

We have re-organized the plant growth section of the methods to be able to understandably describe the details of all plant growth conditions (lines 547 to 565 and 575 to 581 of first submission).

We modified the section explaining the choice of conditions for microarray experiments (lines 565 – 574 of first submission) to make the point more effectively.

We corrected the diagram in Fig. 1A.

We added the detail of the transfer to the figure legend of Fig. 1A-C and S1B.

Point 6. Figure 2C shows the RTL (% UBQ10) for *ZIP4* and *MTP2* in roots of 4-week-old plants, and at -Zn condition they have similar RTL values (close to 30). Even though the RTL cannot be completely comparable between different genes, this seems a little puzzling, because in Fig 2D, where plants grow for 2 additional weeks at -Zn, the RTL values are 10 times different between the two genes, perhaps more in accordance with their different prom::GUS staining patterns and intensities at -Zn shown in Fig 1C and Fig 2B, respectively.

RESPONSE: Again, very observant. We checked all original data and calculations entirely, and we fixed some issues of the axis titles concerning whether % or ‰ are shown relative to the constitutive control gene in RT-qPCR figures. However, we were not able to find any error that caused the remaining discrepancy between Figure 2C and 2D highlighted by the reviewer. The experiments were done at different times in different growth chambers by different experimenters. For hydroponic cultivation, concentrated Hoagland's solution was used in Fig. 2C, whereas 0.25-strength Hoagland's solution was used in 2D. We believe that this can explain the differences between results. The qualitative change in transcript levels dependent on Zn status was consistent between all settings, but the quantitative expression levels of a given Zn deficiency-responsive gene, also relative to that of another Zn deficiency-responsive gene was different between the different settings. These differences between experiments and set-ups do not affect our conclusions.

Changes made:

We clarified these experimental differences in the reorganized methods (see reviewer

#1, 7).

We are now briefly discussing these differences, by adding, after line 263 in the results "Expression levels of Zn-deficiency responsive genes were overall lower in this experiment (Figure 2D), in which we cultivated plants in 0.25x Hoagland's solutions, than in the Zn re-supply experiment, during which we cultivated plants in 1x Hoagland's solution (Figure 2C)."

We corrected the axis labels and axis titles of RT-qPCR figures as appropriate.

Reviewer #2:

The manuscript "The systemic Zn deficiency response" is important.

The following issues require authors attention:

Point 1. Abstract. As in the title the focus is on HMA2 and MTP2 the abstract should be re-organized so that the first part of it deals with these two proteins (HMA2 and MTP2) rather than Zn transcription factors and ZIPs.

RESPONSE: Thank you for your suggestion. The abstract has been re-organized accordingly. Following an editorial comment, we are additionally placing more emphasis on what are the new findings in our manuscript.

Point 2. Introduction is extensively long; it should be shortened and focused. The first 2 paragraphs (lines 63-90) can be significantly condensed and paragraph regarding Fe (lines 128-138) is not required in the introduction (it would be better suited in the discussion).

RESPONSE: Changes made:

- Shortened lines 63-90.
- Moved lines 128-138 to discussion and shortened.

Point 3. Can the authors explain in the manuscript why transcript level of *ZIP4* and *ZIP9* for *hma2 hma4* mutant under Zn deficiency was considered irrelevant to be performed or to be shown? Especially because the *ZIP9* transcript level in the shoots of *hma2 hma4* mutant under control conditions, although higher than in WT plants does not display the same increase as in the shoots of WT plants grown under Zn deficiency conditions.

RESPONSE: Data for *ZIP4* and *ZIP9* transcript levels in *hma2hma4* under –Zn are missing in Fig. 1B, because this experiment/panel is shown to validate our experimental design for comparisons of microarray data, in which we avoided sick/dying plants. The purpose of this figure is to illustrate that *hma2hma4* shoots grown under control conditions are physiologically Zn-deficient like shoots of a wild-type plant grown on Zn-deficient medium, but not the roots. As for the difference in *ZIP9* transcript levels between shoots of the wild-type grown under Zn deficiency and shoots of *hma2 hma4* grown under control conditions, we believe that an approximately 2-fold difference in transcript levels is a small difference by comparison to other differences observed in the experiment shown in this figure, and it is fully consistent with Figures 1A and S1B. Thus, we feel that discussing this minor difference extensively would distract readers from the highly relevant observations of major differences.

Point 4. Results. (a) Can the authors explain the difference in the transcript level of MTP2 in the roots of WT and *hma2 hma4* under control and also under -Zn conditions (Fig. 2A). (b) And how does this correlate with the lack of visible staining in the roots of ProMTP2:GUS in the *hma2 hma4* mutant background (Fig. 2B)?

RESPONSE: (a) We hope we understand what is meant by the comment on Fig. 2A. Sure we can explain the differences in transcript levels of *MTP2* in the roots of WT and *hma2 hma4* under control and also under -Zn conditions (Fig. 2A): They confirm both the microarray data and our expectations. *MTP2* transcripts are only detectable in roots of Zn-deficient wild-type plants and not in roots cultivated under control conditions. The fact that *MTP2* transcript levels are higher in *hma2hma4* roots than in the wild type under both control and – Zn growth conditions supports our idea of systemic upregulation of root *MTP2* transcript levels in dependence on shoot physiological Zn deficiency. (b) There was visible GUS staining in the roots of ProMTP2:GUS in the *hma2 hma4* mutant background (Fig. 2B).

Changes made:

We added a new sentence in results (Line 226, after "(Figure 2A)"): This result supported our hypothesis that *MTP2* transcript levels in the root respond to the physiological Zn status of the shoot and not the Zn status of the root (see Figure 1B).

We added an extra arrow to point out GUS histochemical staining in the lower right panel of Fig. 2B.

Point 5. Results. Can the authors also explain the difference in the level of expression of *ZIP4* in roots of WT plants under Zn deficiency at 19-days (Fig. 1B) and at 4 weeks (Fig. 2C). Transcript level of *MTP2* is upregulated in roots of WT under -Zn, but it seems to be relative constant with the age (19-days (Fig. 2A) and 4 weeks (Fig. 2C)).

RESPONSE: In our opinion, it is not possible to conclude on the dynamics of gene expression during Zn deficiency, i.e., on constant or changing expression of Zn-deficiency response genes from comparisons between 19- and 21-d-old seedlings grown on agar- solidified media and 8-week-old plants grown in the hydroponic system. This is because of the entirely different growth regimes in the different experiments. Hydroponically grown plants represented in Figure 2C were only transferred to - Zn solutions three weeks before harvest, whereas 19-day-old plants shown in Fig. 2A were grown on - Zn agar-solidified media from germination on, and 21-d-old plants shown in Fig. 1B were transferred from 0.5x MS media onto - Zn agar-solidified treatment media 7 d after germination. The different growth regimes were chosen because of different purposes of the experiments and different practical requirements. Figures 1A-C were done to validate the principle of our microarray-based identification of candidate genes for systemic regulation, in which we aimed at only moderate Zn deficiency to prevent plant death-

associated changes in gene expression. Figure 2A was done to confirm the regulation of one identified candidate gene in a different experiment, where there was no strong need to moderate the degree of Zn deficiency in *hma2hma4*. Re-supply is less disruptive in hydroponics than in plate culture (Figure 2C). We found errors in the axis titles (units chosen), which after correction, lead to very similar *ZIP4* gene expression in roots in Fig. 1B and 2C.

Changes made:

We corrected the legend text for panel 2C, because an important piece of information was lost in its writing. It now reads: "*ZIP4* and *MTP2* transcript levels in roots of 8- week-old hydroponically cultivated WT plants. Plants were transferred to Zn-sufficient (5 µM, Suf.) or Zn-deficient (0 µM added Zn, - Zn) hydroponic solutions three weeks, or Zn (5 µM) was re-supplied to - Zn-grown plants for the indicated periods of time before harvest. "

Point 6. In order to further support their conclusion that MTP2 transcript level is also regulated by signals of the Zn status in the shoot (Fig. 2D), the authors should provide evidence that the reduction of MTP2 transcript in roots of plants sprayed with Zn in comparison to the ones sprayed with mock happens also in the hma2 hma4 background. And similarly for ZIP4.

RESPONSE: We do not agree that it is necessary to prove our point. In addition to the microarray data involving *hma2hma4* and their confirmation, an independent approach for demonstrating systemic regulation in wild-type plants was to show the downregulation of root *MTP2* transcript levels in Zn-deficient plants of which shoots were sprayed with Zn (Fig. 2D). In addition, it was impossible to perform this experiment because *hma2hma4* do not survive until the age at which we initiated spraying under either – Zn or control Zn growth conditions in hydroponics. The double mutant dies after 2 w in hydroponics, and it would require supplementation with higher Zn concentrations than our control solution to survive.

Point 7. Results. Conclusion on lines 297-299 should be rephrased as the statement: mtp2 is impaired in shoot growth is not supported by the results, because, as the authors mentioned, the reduction in shoot biomass is not significant (Fig. 4D).

RESPONSE: Shoot biomass is reduced in Figure 3C (19-d-old). We agree that it was not significantly reduced in 4D. Nevertheless, we feel that our conclusion statement is justified: These details are mentioned in the results, and in the conclusion, we are aiming to make a summary statement without re-iterating the complexity of results from all experimental designs and experiments.

Point 8. Results. Define what would be a representative leaf taken for Chlorophyll content (Fig. 4C), as looking at Fig. 4A under -Zn, only older leaves of *mtp2* mutant seem to show chlorotic whereas young leaves seem to look similar to the ones of WT.

RESPONSE: We never sampled the youngest, nor the oldest, leaf. Instead, we sampled an approximately 'average - sized' leaf from each rosette for the determination of chlorophyll levels. Sixteen individual rosettes should render the results fairly robust

Changes made: We replaced "representative leaf" in the legend of Fig. 4C by "fully expanded leaf of intermediate age"

Point 9. Results. Can the authors explain why a shorter promoter of *AtMTP2* was used to investigate MTP2 localization in planta in comparison to the *MTP2* promoter used for fusion to GUS?

RESPONSE: The promoter-GUS construct included a large upstream fragment, which was unnecessarily long. So when we later made the MTP2-GFP fusion, we included a shorter upstream sequence that we deemed sufficient for simplicity. In addition, we believed that the localization of GUS might be misjudged if the GUS protein is localized in the cytoplasm (i.e., less vacuolated cells would appear to have higher GUS activity, but they only have more cytoplasm). Therefore, we also included in the promoter-GUS construct a part of the *MTP2* coding sequence that encodes the N-terminus and the first two transmembrane helices. We have changed the text in order to include all cloning details correctly.

Changes made:

We replaced from Line 615-618: "To generate the *ProMTP2:GUS* construct, a 2,542 bp long sequence fragment comprising part (722 bp) of the transcribed region of the upstream gene including its 3'-UTR, the 1,263-bp intergenic region, the 183 bp long 5'-UTR of *MTP2*, an intron of 93 bp in length just before the start of the

coding sequence and 281 bp of the coding sequence of *MTP2*, which encode the N-terminus including the first two transmembrane helices, was amplified from genomic DNA, introducing *NheI* and *NgoMIV* sites using the primers MTP2prom_for and MTP2prom_rev. The PCR product was cloned into the *XbaI* and *XmaI* sites of pGPTV- kan (DNA Cloning Service, Herman Schmidt, Hamburg, Germany)."

We replaced in line 622 from "including ... MTP2" by "including 1,277 bp upstream of the *MTP2* coding sequence"

We made a few small formal improvements: italicized "*MTP2*" in line 623 and renamed the primers in lines 623 and 624 by replacing "CDF2" with "MTP2" in the primer names.

Point 10. Results. Provide information regarding the media on which the plants were grown for Fig. S4B-D and provide explanation for the difference in the fresh biomass between 19-days old plants used to generate Fig. 3C and the 17-days old plants in Fig. S4B. Add legend Ctrl and -Zn on the chart in Fig. S4B.

RESPONSE: To be able to determine root length before the roots reach the bottom of the vertical plate, we harvested plants at 10 d of age in Figure S4.

Changes made: The legend of Figure S4 was corrected accordingly.

Point 11. Results. (a) Make it evident either in the text or in the Figs legend if the control Zn conditions are different between the 19-days old seedlings used to generate Fig. 3E and Fig. 7A as the Zn partitioning is twice higher in control conditions in Fig. 7A compared to Fig. 3E. (b) Also for consistency, use Zn partitioning not Zn concentration in Fig. 7 legend. For consistency, if control Zn and -Zn are the same as in the other Figs do not mention in brackets the concentrations. Mention also in the Fig. 7 legend the media the plants were cultivated.

RESPONSE: (a) The control Zn concentrations were not different between Figure 3E and 7A. We do not know why Zn partitioning differed under control conditions (not under– Zn conditions) between the two experiments conducted in the same facilities more than a year apart. Note that partitioning is a highly derived parameter that depends on both shoot and root biomass, as well as leaf Zn concentration. Less derived parameters are given in Suppl. Data for Fig. 3. We decided to show Zn partitioning, because it is physiologically the most sensible to do in the context of the biological question asked here. In addition, conclusions would be no different when using shoot Zn concentrations (Figure S3C) or shoot:root ratios of Zn concentrations (Figure 3E) instead.

Changes made:

Changed 'concentration' to 'partitioning' and '16-8 seedlings',to '16-18 seedlings' in legend of Figure 7.

Removed actual Zn concentration values ("5 µM added Zn", "0 µM added Zn") as suggested by the reviewer for consistency.

Added to legend of Figure 7, "agar-solidified" before "media" (changed from "medium").

Point 12. Discussion. (a) Provide discussion to explain how would the MTP2 and HMA2 effect be additive in the light of their localization in planta. (b) The function of MTP2 in planta is speculative as GUS staining within the elongation zone (where cells are not yet fully differentiated) of the lateral roots does not support the supposition of its implication in movement of Zn toward the xylem for loading. (c) And direction of Zn transport through MTP2 is speculative. There CDFs (like MTP2) e.g. *Saccharomyces cerevisiae* MSC2 mediates export of cations from nucleus to the cytosol (Li and Kaplan, 2001, JBC), and ScMMT1 and 2 might function as mitochondrial Fe exporters (Li and Kaplan, 1997, JBC).

RESPONSE: (a) We believe that this is discussed (lines 470 to 502) in our first submission and visualized in Figure 8. (b) Fig S1E clearly shows that in extended -Zn deficiency we did observe GUS staining above the elongation zone (see also text of first submission lines 232 to 236). In addition, the protein is present also subsequent to promoter activity (i.e., in older cells further up the root)(Shown in Figure S1G of first submission, bottom left panel; now S1H) (c) More recent publications (Ellis et al., 2004, 2005) provide a far more advanced characterization of the function of yeast MSC2, which strongly supports our working model. This is discussed in detail in the first submission (first submission lines 434 to 444).

Changes made:

We modified the corresponding section in the abstract, which was somewhat misleading in an effort to keep it short.

The model in Figure 8 was altered to reflect more accurately the overlap of the zones of *MTP2* and *HMA2* expression. The sentence that was in lines 53-55 of the first submission now reads: "Chimeric GFP fusion proteins of MTP2 complement an *mtp2* mutant and localize in the ER membrane of the outer cell layers from elongation to root hair zone of lateral roots." Note that the abstract was also rearranged.

TPC2018-RA-00207R1 2nd Editorial decision – *acceptance pending* July 24, 2018

We are pleased to inform you that your paper entitled "Systemic upregulation of MTP2- and HMA2-mediated Zn partitioning to the shoot supplements local Zn deficiency responses of Arabidopsis" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor August 24, 2018