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Review

Metals and seeds: Biochemical and molecular implications and their significance for seed germination☆

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ABSTRACT

Seeds contain the embryo as a new plant in miniature and have two major functions, reproduction and dispersal. Seed formation completes the process of plant reproduction and, with seed germination, the next plant generation starts. Given the ever-increasing environmental pollution with metal(loid)s, it is perhaps surprising that relatively few reports detail the impacts of metals on seed metabolism, viability and germination in comparison to the numerous publications on the effects of metals in vegetative tissues, particularly roots and shoots. This review provides information on metal(loid) homeostasis, detoxification and tolerance in relation to seed metabolism and performance. The delivery of metals from the mother plant into seeds and their implications on seed development are discussed, as are their uptake upon seed imbibition and subsequent effects on seed germination. Implications for seeds and seedlings on the biochemical and molecular level are discussed and finally, applied aspects are considered regarding the use of seeds for soil and water purification, and in phytoremediation programmes. We conclude with a perspective on future metal research in relation to seed biology.

Contents

Abbreviations: AGE, advanced glycosylation end-products; APX, ascorbate peroxidase; AsA, ascorbic acid; CAT, catalase; CAX, cation exchanger; CDF, cation diffusion facilitator; DGDG, digalactosyldiacylglycerol; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; HMA, heavy metal ATPase; IRT, iron-regulated transporter; MDA, malondialdehyde; MT, metallothionein-like; MGDG, monogalactosyldiacylglycerol; micro-PIXE, micro-proton-induced X-ray emission; NA, nicotianamine; NO, nitric oxide; Nramp, natural resistance-associated macrophage protein; OPT, oligopeptide transporter; PEP, phosphoenolpyruvate; POX, peroxidase; PR, pathogenesis-related; RNS, reactive nitrogen species; ROS, reactive oxygen species; $O_2^{\bullet-}$, superoxide; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TG, total germination; VIT, vacuolar iron transporter; YSL, yellow-stripe-like; ZIF, zinc-induced facilitator; ZIP, ZRT-IRT-like protein; ZRT, zinc-regulated transporter.

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1. Metal uptake and transport to seeds

The delivery of metals to seeds depends upon uptake by the mother plant, and subsequent transport to developing seeds. Due to the potential toxicity of excess levels of metals, their uptake and distribution and the intracellular concentration must be carefully regulated. Membrane transport systems and gene families involved in metal homeostasis include heavy metal ATPases, natural resistance-associated macrophage proteins (Nramps), cation diffusion facilitators (CDFs), the ZRT (zinc-regulated transporter)-, IRT (iron-regulated transporter)-like protein (ZIP) family and cation antiporters. Most of the gene families in plants are large compared to analogous families in other organisms, reflecting the need for plants to respond to fluctuating environmental conditions and metal availability with low and high affinity systems, and the need to control metal transport at the cellular and organellar level (Hall and Williams, 2003). The uptake of essential metals from the soil and subsequent transfer to the xylem for transport to other plant parts has been reviewed by Briat and Lobreaux (1997) and Colangelo and Guerinot (2006). Here, we will deal with the transport of metals to developing seeds, the tissue-specific localisation of metals within seeds, and finally the uptake of metals from soil by intact seeds.

1.1. Metal delivery to seeds

Metals are normally found at the highest concentrations in the roots, and at the lowest concentrations in the reproductive tissues (Malan and Farrant, 1998; Shanker et al., 2005). This is because metals are sequestered into the vacuoles of root and shoot tissue and the subsequent availability of free metals in the symplast can be low. For example, concentrations of Cd and Ni in soybean were 30 and 20 times, respectively, higher in roots than in leaves. Cadmium concentration was lowest in the seeds, whereas the concentration of Ni was the same in both leaves and seeds (Malan and Farrant, 1998).

Essential metals, e.g. Fe, need to be transported to the developing seeds (Fig. 1). Mineral transport to seeds is believed to occur solely via the phloem, because movement of the xylem sap is driven by transpiration, which is limited by the impermeability of the seed coat as demonstrated in soybean (*Glycine max*) (Laszlo, 1994; Stacey et al., 2008). Radiotracer experiments of phloem loading of Fe in *Pisum sativum* showed that Fe delivery to the seed occurred throughout the period of seed development. The predominant source of Fe is through continuous uptake via the roots rather than mobilisation of stored reserves from other tissues, which supplies about 20–30% of seed Fe (Grusak, 1994). Similarly, remobilisation of reserves from senescing leaves has been estimated to supply at most 40% of the metal content of Arabidopsis seeds (Curie et al., 2009). In *Pisum sativum*, phloem loading was observed in the leaflets, stipules and pod walls, with other potential sites of phloem loading being the stem, petiole and peduncle (Grusak, 1994). The distribution of Fe to developing seeds via the phloem is hindered by pH values greater than 7 at which Fe precipitates. Nicotianamine (NA) is a strong chelator of Fe(II) and is abundant in the phloem sap (Sharma and Dietz, 2006). The Fe(II)–NA complex is very stable at pH 7 and above, and it is likely that Fe(II) is transported via the phloem as a NA chelate. Tomato and tobacco mutants deficient in NA show symptoms of chlorosis and failure to flower or production of sterile flowers as a result of disrupted Fe delivery to developing organs (Ling et al., 1999; Takahashi et al., 2003). An Arabidopsis mutant with low NA synthase activity also developed chlorotic leaves, and in addition the seeds accumulated less NA and Fe than wild-type seeds, showing that NA is needed for Fe supply to developing seeds (Klatte et al., 2009). Nicotianamine can also form stable complexes with Cu, Fe, Zn, Ni, Co and Mn to facilitate transport of metals via the phloem (Curie et al., 2009). Deoxymugineic acid, which is a derivative of NA, has been identified in the phloem sap of *Oryza sativa*, and Fe may be delivered as a Fe (III)-deoxymugineic acid complex in this species (Grusak, 1994).

There is no vascular link between the maternal tissues of the seed coat and the developing embryo. Nutrients are unloaded from the phloem and translocated into the embryo apoplastically, prior to symplastic distribution. Metals can apparently not cross the apoplastic barrier and therefore are excluded from the embryonic tissues (Vogel-Mikus et al., 2007) unless specific transporters are used. Reverse genetics studies using Arabidopsis mutants with loss of function of yellow-stripe-like (*ysl*) transporter genes provided evidence that AtYSL1 is involved in the delivery of Fe to the seeds as a Fe–NA complex (Le Jean et al., 2005). It was proposed that *YSL* genes play an important role in transferring metals from an apoplastic to a symplastic path (Curie et al., 2009). Another transporter implicated in Fe delivery to seeds in Arabidopsis is AtOPT3, which belongs to the oligopeptide transporter (OPT) family and appears to be required for long-distance transport of Fe via the phloem, and also for transport across apoplastic borders between tissues in developing seeds (Stacey et al., 2008).

The transport of Zn to developing barley grains has been studied using laser capture microdissection to isolate cell sections of the phloem unloading region, aleurone layer, endosperm and embryo followed by microarray analysis of gene expression in these cell sections. Transfer cells form the interface between the symplast of the maternal phloem in the nucellar projection and the apoplast of the endosperm cavity. It was proposed that Zn is unloaded from the phloem as Zn–NA complexes into transfer cells where it is transiently stored in the vacuoles before being pumped into the endosperm apoplast. Transporters mediate uptake of Zn–NA into aleurone cells, and Zn is then distributed symplastically to cells of the endosperm and embryo. Transporters required in the trafficking of Zn from the phloem to the developing grain include members of the P_{1B}-type heavy metal ATPase (HMA), ZIP, CDF, Nramp, vacuolar iron transporter 1 (VIT1), cation exchanger (CAX), zinc-induced facilitator 1 (ZIF1) and YSL protein families (Tauris et al., 2009).

Non-essential metals are also transported to seeds, and this represents a problem in crop species, where the levels of metals present in seeds can exceed safe limits for human consumption. For example, Cd is transported to developing seeds of Indian mustard (*Brassica juncea* L. Czern) via the phloem as a result of a shift in the source-sink relationship as seeds fill and draw increasing levels of photosynthate during the process of seed set. Cadmium has been proposed to enter the phloem through two possible mechanisms: direct transfer from the xylem in stems or rapid export from the leaves before the Cd is compartmentalised into the vacuole (Sankaran and Ebbs, 2008). Two rice cultivars with different seed Cd