Metal immobilization: where and how?

Stéphane Mari and Michel Lebrun

Abstract

Metal immobilization away from metabolically active sites within the cell represents the last step in both the homeostasis of metals and the detoxification of metal in excess. Assessment of the importance of this step requires having access to the *in vivo* speciation of metals. Evolving techniques have made it possible to acquire more reliable *in situ* profiling of: *(i)* spatio-temporal accumulation of metal, *(ii)* characterization of the metal-ligands complexes and determination of the structure of the different bio-ligands involved. The chapter "metal immobilization: where and how?" presents the role of different metal-chelators in plants, based on examples from works using non-invasive techniques and genetic approaches at both the whole plant, cellular and subcellular levels. The aim of the chapter is to give a survey of the key molecules and processes involved in metal immobilization in plants, on the basis of direct and robust evidences of the *in vivo* speciation of metals.

1 Introduction

The last step of heavy metal detoxification is the immobilization, in chemical forms as inactive as possible, with the aim of protecting metabolically active cells. By far, the mechanisms controlling the metal immobilization have been the least studied, when compared to metal ion uptake and transport in the plants. The difficulties to address this question lay on a major technical challenge: the need to develop and adapt non-invasive and non-destructive techniques aimed at identifying precisely the localization of metal ions within a tissue and the ligands involved in their chelation. As a consequence, few mechanisms have been identified so far, limiting the targets for molecular and genetic approaches.

The permanent concern, when using for example mass spectrometry approaches on extracts from metal-enriched plant tissues, is the artefactual formation of metal complexes by mixing high affinity chelators with metal ions that were in different cell compartments or tissues, leading to conclusions far from the *in vivo* situations.

This chapter deals with metal chelators, the different categories, their capacities, and their relative role in metal chelation. However, it has to be kept in mind that in this field the main limitation lays on the development and improvement of non-invasive techniques allowing the direct visualisation of the metal speciation *in*

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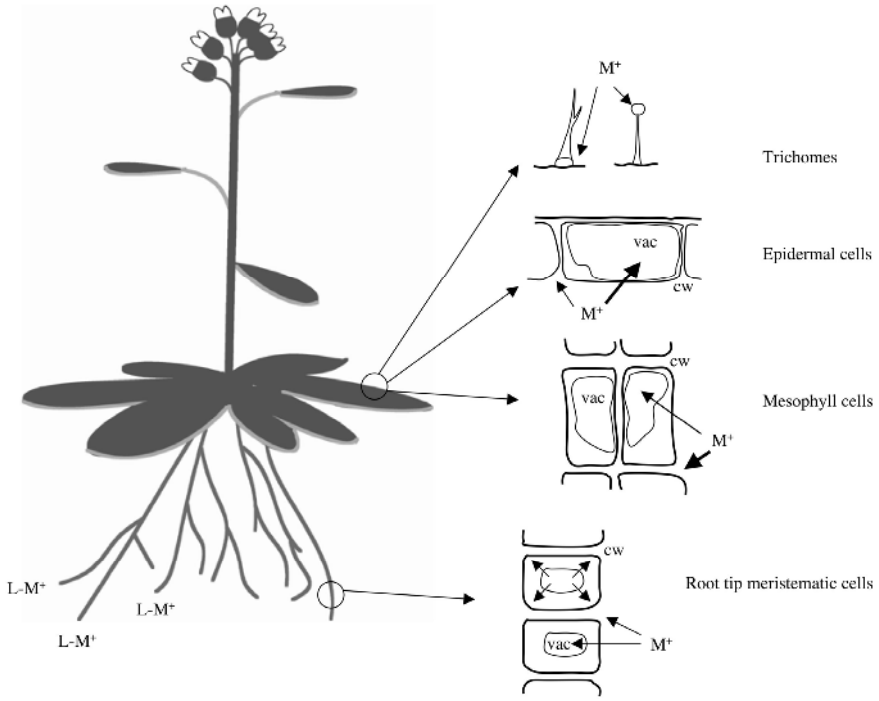

Fig. 1. Synthetic view of the mechanisms involved in metal immobilization. L-M^+ represents the complexation of metal ions in the rhizosphere (Section 1); following uptake by the root system, metal ions are bound to the cell wall compartment and induce an increased vacuolisation, in meristematic root cells (Section 2); in the leaves two different strategies have been identified from works on hyperaccumulator species: (*i*) a massive accumulation of metals in the vacuole of epidermal cells and a lower immobilization in the cell wall, (*ii*) the concentration of metals in the basal part of trichomes and a distribution of the remaining metal ions in the mesophyll cells with a relatively higher binding to the cell wall, compared to the vacuole. In the non-accumulator species studied, metals in the leaves are concentrated in the trichomes (Section 3). Abbreviations: L-M^+, metals complexed in the rhizosphere; M^+, metal ions; cw, cell wall, vac, vacuole . The thickness of the arrows are indicative of the relative amount of metals accumulated in the corresponding compartment.

situ and *in vivo*, to avoid artefacts created by decompartmentation. The examples chosen to illustrate our purpose (see Table 1 for a compilation of the examples cited in this section) will, therefore, come from works based on (*i*) non-invasive approaches coupled to electron/atomic spectroscopy, (*ii*) histo and cytological studies allowing the identification of organelles/cell types/tissues/specific organs involved in metal chelation, (*iii*) genetic approaches with mutants affected in the synthesis of potential chelators.