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Interaction Between Heavy Metals and Thiol-linked Redox Reactions in Germination

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Abstract: Thioredoxin (TRX) proteins perform important biological functions in cells by changing the redox state of proteins via dithiol disulfide exchange. Several systems are able to control the activity, stability, and correct folding of enzymes through dithiol/disulfide isomerization reactions including the enzyme protein disulfide-isomerase, the glutathione-dependent glutaredoxin system, and the thioredoxin systems. Plants have devised sophisticated mechanisms to cope with biotic and abiotic stresses imposed by their environment. Among these mechanisms, those collectively referred to as redox reactions induced by endogenous systems. This is of agronomical importance since a better knowledge of the involved mechanisms can offer novel means for crop protection. In the plant life cycle, the seed and seedling stages are key developmental stages conditioning the final yield of crops. Both are very sensitive to heavy metal stress. Plant redox reactions are principally studied on adult plant organs and there is only very scarce informations about the onset of redox regulation at the level of seed germination. In the here presented study, we discussed the importance of redox proteins in plant cell metabolism and defence. Special focus is given to TRX, which are involved in detoxification of ROS and also to their targets.

Key words: Germination, heavy metals, redox, seeds

INTRODUCTION

All organisms contain complex regulatory machinery to maintain the redox status of -SH groups in proteins and low molecular weight sulphydryls. Glutaredoxins (GRX) and thioredoxins (TRX) are small heat-stable oxidoreductases which contain two conserved cysteine residues in their active sites (Rouhier *et al*., 2001). They were originally identified as hydrogen donors for ribonucleotide reductase, but are also required for a number of antioxidant and metabolic enzymes that form a disulphide as part of their catalytic cycle (Jacquot *et al*., 2009). The glutathione (GSH) is generally considered as having a unique cellular redox buffering function, capable of maintaining reduced the thiol-redox cellular balance, and also to eliminate electrophilic compounds including Reactive Oxygen Species (ROS). This function is in keeping with the characteristics of GSH of being the most abundant low molecular thiol of eukaryotic cells and having a relatively low redox potential (Rouhier *et al*., 2008).

Heavy metal stress: Although heavy metal is not essential for plant growth, its mobility in soil-plant system allows its easy entry into plants, where it may cause toxic effects on plants, animals and human health through food chain (Warner, 1993). Studies carried out in different plant species have revealed that Cd is strongly phytotoxic and causes inhibition in growth (Smiri *et al*., 2009). One recognized explanation of the impact of heavy metals on the plant physiology is that it results in several nutritional disturbances (Fig. 1). The presence of Cd in plants results in many physiological alterations affecting both nitrogen and carbohydrate metabolism (Chaffei *et al*., 2004). The most sensitive responses of higher plants to Cd are the decrease in transpiration and overall inhibition of photosynthesis (Greger and Johansson, 1992; Chugh and Swahney, 1999). Cadmium induces alterations in the functionality of membranes, by triggering changes in their lipid composition (Howlett and Avery, 1997). It was shown that Cd-induced toxicity involves some other senescence-like processes, such as the participation of oxidative stress, mediated by H_2O_2 and leading to gradual increases in activities of certain antioxidant enzymes, such as catalase and peroxidase, along with increased lipid peroxidation (Chaoui *et al*., 1997) and proteolytic degradation (Romero-Puertas *et al*., 2002). Cadmium also induces peroxisome-senescence symptoms in leaves with the induction of the glyoxylate cycle enzymes, malate synthase and isocitrate lyase, as well as peroxisomal peptidases (McCarthy *et al*., 2001), enzymes that are also known as leaf senescence-associated factors (Buchanan-

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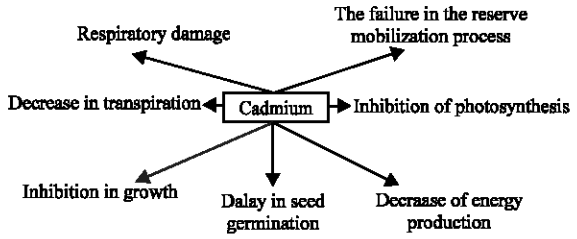

Fig. 1: Representation of the most sensitive responses of higher plants to cadmium

Wollaston *et al*., 2003). Seed germination represents a limiting stage of the plant life cycle under heavy metal stress situations because it is the first interaction with the polluted environment (Claire *et al*., 1991; Warner, 1993). When medium surrounding the seed is contaminated with Cd, delays in germination are often observed (Claire *et al*., 1991; Chugh and Sawhney, 1996; Munzuroglu and Geckil, 2002; Mihoub *et al*., 2005). This can be associated with several disorders in the event chain of germinative metabolism. The inhibition of seed germinations after exposure to cadmium is the consequence of a failure in the reserve mobilization process. Excessive accumulation of Cd in cotyledons compromises the seeds germination through a negative interference of pollutants with mineral and organic reserves mobilization. Metal treatments induce a pronounced restriction in transport of micronutrients and of soluble sugars from cotyledons, and in the freeing of amino acids (Mihoub *et al*., 2005; Rahoui *et al*., 2008), followed by the decrease of α-amylase and acid phosphatase activities. The impact of Cd on the seed germination is that it produces respiratory disturbances (Chugh and Sawhney, 1996; Bansal and Sharma, 2000; Bansal *et al*., 2002; Smiri *et al*., 2009), results in a decrease of ATP production.

Plant redox systems: Thioredoxins occur in most all organisms. Although only one type of thioredoxin has been detected in bacteria and animals, three well-characterized variants exist in photosynthetic cells. The thioredoxins m and f are located in chloroplasts (Traverso *et al*., 2008; Chibani *et al*., 2009). Plants have a third class of thioredoxin, the h type-located in the cytosol, endoplasmic reticulum and mitochondrion (Reichheld *et al*., 2002; Montrichard *et al*., 2003; Gelhaye *et al*., 2005; Traverso *et al*., 2007). The oxidized form of each thioredoxin contains a disulfide (-S-S-) bridge that is reduced to the sulfhydryl (-SH) level by either reduced ferredoxin or NADPH. The chloroplast thioredoxins are reduced by electrons from excited chlorophyll via ferredoxin and ferredoxin-dependent thioredoxin reductase (FTR) (Balmer *et al*., 2006). The thioredoxin h is reduced by NADPH in a reaction catalyzed by a flavoprotein enzyme, NADPH-dependent thioredoxin reductase (NTR) (Jacquot *et al*., 1994, 2009). The reduced form of TRX is an excellent catalyst for the reduction of intramolecular disulfide bonds of proteins that are only slowly reduced by GSH or GRX, the other major cellular sulfhydryl reductants (Rouhier *et al*., 2002a, b) (Fig. 2). The fructose-1, 6-bisphosphate is preferentially activated by thioredoxin f (Pagano *et al*., 2000; Traverso *et al*., 2008). L'ATP synthase, which catalyzes the synthesis of ATP in photophosphorylation, is activated by both chloroplast TRX (Balmer *et al*., 2006). TRX m effectively activates NADP:malate dehydrogenase, an enzyme of the C_4 photosynthetic carbon cycle and C_3 redox shuttle, and deactivates glucose-6-phosphate dehydrogenase (Pagano *et al*., 2000). TRX m has been found to regulate other light-dependent chloroplast processes, such as the translation of mRNA. The TRX are known to play a broad regulatory role in many cell types (Balmer *et al*., 2006). The ferredoxin-thioredoxin system activates enzymes of chloroplast processes. Thus, the C_4 cycle, photophosphorylation, and mRNA translation are activated by TRX via NADP: malate dehydrogenase (Pagano *et al*., 2000; Balmer *et al*., 2006). In addition to its role in C_4 plants, TRX-linked NADP: malate dehydrogenase functions in C_3 plants in a cyclic process that transports malate synthesized in the chloroplast to the cytosol, where it generates NADH. The main regulatory enzyme of the oxidative pentose phosphate cycle, glucose-6-phosphate dehydrogenase that functions in the opposite way than the enzymes of photosynthesis, is deactivated upon reduction by TRX (Pagano *et al*., 2000). By lowering the activity of this enzyme, light inhibits operation of the oxidative pentose phosphate pathway for the breakdown of carbohydrate. Chloroplasts thus use TRX to link light to the control of carbon flow through two major opposing carbon pathways (Schurmann and Jacquot, 2000). The function of TRX h is currently being investigated in several plant systems, including seed (Lozano *et al*., 1996; Ishiwatari *et al*., 2000; Reichheld *et al*., 2002; Montrichard *et al*., 2003; Traverso *et al*., 2007). It has been found to promote the mobilization of carbon and nitrogen of the endosperm early in grain germination (Montrichard *et al*., 2003). TRX h appears to act (1) by reducing the major seed storage proteins, there by enhancing their susceptibility to proteases, (2) by activating a newly discovered type of calcium-linked

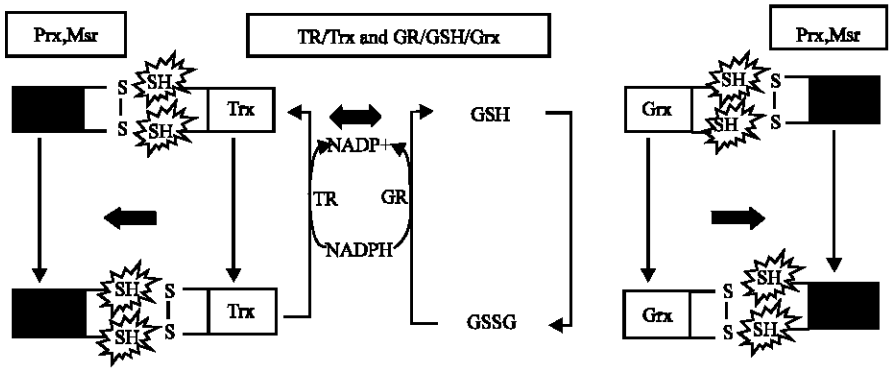

Fig. 2: Representation of the interactions between thioredoxin (TRX/NTR), glutaredoxin (GRX/GR/GSH) systems and targets: peroxiredoxins (PRX) and methionine sulfoxide reductase (MSR)

protease (thiocalsin) and (3) by neutralizing low-molecular-weight proteins that inhibit enzymes of starch degradation (Yano *et al*., 2001). TRX h has been identified as a prominent component of phloem (Ishiwatari *et al*., 2000). In plant cells, the production of ROS such as superoxide anion radical ($O_2^{\bullet -}$), the hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) takes place in chloroplasts, mitochondria, peroxisomes, the plasma membrane and the apoplastic space. All biologically relevant macromolecules, i.e., nucleic acids, membrane lipids and proteins, are susceptible to damage by ROS. Reactive oxygen species are involved in various aspects of seed physiology, where they support dual function being either cytotoxic or playing a role in development, dormancy breakage and in defense against biotic and abiotic stresses (Bailly, 2004; Finch-Savage and Leubner-Metzger, 2006). Efficient flux through plant electron transport cascades requires the simultaneous presence of both oxidized and reduced forms of electron carriers. This requirement, known as redox poising, involves a continuous flux of electrons to molecular oxygen from multiple sites in the photosynthetic and respiratory electron transport chains (Navrot *et al*., 2007). Apart from the specialized water producing reactions catalyzed by specific oxidases, the initial product of this flux is superoxide, from which other ROS are subsequently produced. Singlet oxygen is also formed during light capture and photochemistry. Numerous enzyme systems produce superoxide or H_2O_2. The reactive nature of these intermediates means not only that their accumulation must be controlled but also that they are able to act as signaling molecules (Navrot *et al*., 2007). Oxidative stress is defined as the increase of ROS to levels that disrupt the cellular redox homeostasis. Sub-cellular compartmentation of defence mechanisms is required for efficient removal of ROS at their generation sites. Every sub-cellular compartment has an own protection system consisting of specialized antioxidative enzymes such as catalase, ascorbate peroxidases, glutathione peroxidase, glutathione reductase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), superoxyde dismutases (SODs), the Peroxiredoxins family (Prx) and metabolites of low molecular weight such as ascorbate, glutathione and tocopherol (Rauser and Meuwly, 1995; Rouhier *et al*., 2002a; Chaoui and Ferjani, 2005). During seed formation, desiccation damage is largely suspected to be related to oxidative processes, as indicated by the observation that dry, mature, desiccation-tolerant seeds display high levels of ROS-scavenging enzymes (e.g., catalase and glutathione reductase) as well non enzymatic antioxidant components such as glutathione and tocopherols (Bailly, 2004).

The description of several thioredoxin (TRX)-related antioxidant systems in phloem, including glutaredoxins (GRX) or peroxiredoxins (PRX) (Szederkenyi *et al*., 1997; Rouhier *et al*., 2001; Walz *et al*., 2002), suggested the involvement of TRX in the mechanism of ROS detoxification (Reichheld *et al*., 2002; Traverso *et al*., 2008). In addition, it has recently been proposed that TRX in vascular veins could serve as a long-distance thiol signal between different parts of the plant (Balmer *et al*., 2006). Methionine residues are readily oxidized by ROS to form methionine sulphoxides (MetSO), which is reduced to methionine by methionine sulphoxide reductase (MSR) (Romero *et al*., 2004). This system acts as an antioxidant, repairing proteins damaged by oxidative stress (Moskovitz *et al*., 1997). TRX f and m-type interact in vivo with the yeast methionine sulphoxide reductase in agreement with results showing the interaction between methionine sulphoxide reductase and TRX in *Arabidopsis* (Vieira Dos Santos *et al*., 2005). The yeast complementation test suggests the involvement of pea TRX m and f in the mechanism of peroxide detoxification (Traverso *et al*., 2008). In addition, the antioxidant effect

of pea TRX m and f might be due to their capacity to reduce other types of peroxiredoxin (PRX). Regarding gene expression analysis in response to oxidative stress in pea plants, PsTRXf and PsTRXm transcripts increased in roots of plants subjected to hydrogen peroxide treatment (Pagano *et al*., 2000; Traverso *et al*., 2008). A similar behaviour was described for a TRXm in the green algae *C. reinhardtii*, showing no change in its protein level although it displayed an increase of gene expression in response to heavy metals.

Accumulation of ROS and free radicals has often been considered as one of the most important factors of seed ageing (Hendry, 1993; McDonald, 1999). On imbibition under favourable conditions, an inert quiescent seed is transformed into a vigorously metabolizing system. This transition entails development of various biochemical capabilities in a programmed, finely controlled and co-ordinated manner (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). Antioxidant statu (Wojtyla *et al*., 2006), appears to set the threshold for general plant defense responses, particularly those provoked by biotic stresses. Indeed, modulation of the ROS-antioxidant interaction plays a part in many stresses, as well as other responses to the environment, and in the regulation of plant development (Yano *et al*., 2001; Wojtyla *et al*., 2006; Posmyk *et al*., 2009). Thus, a number of studies have documented the production of H_2O_2, nitric oxide, hydroxyl radicals, and superoxide radicals during germination of various species (Leprince *et al*., 1990; Puntarulo *et al*., 1991; Cakmak *et al*., 1993; Aalen, 1999; Bailly, 2004). Such oxidative damage accumulates over time during the life cycle of many organisms and has been suggested to be one possible cause of aging (Stadtman, 1992; Agarwal and Sohal, 1994). During seed germination, heavy metal damage is also largely suspected to be related to oxidative processes, as indicated by high levels of ROS-scavenging enzymes (Posmyk *et al*., 2009; Vazquez *et al*., 2009) as well nonenzymatic antioxidant components such as glutathione and tocopherols (Aina *et al*., 2007; Posmyk *et al*., 2009). The presence of all antioxidative enzymes as well as molecular antioxidants in dry seeds allowed the antioxidative machinery to be active as soon as the enzymes were reactivated by seed imbibition (Yano *et al*., 2001; Bailly, 2004; Finch-Savage and Leubner-Metzger, 2006). Seeds scavenge (ROS) by detoxification mechanism provided by an integrated system of enzymatic antioxidants and also non-enzymatic antioxidants. The changes in free radical levels, antioxidant contents and enzymatic activities appear to be more closely related to metabolic and developmental processes associated with preparation for germination (Leprince *et al*., 1990; Puntarulo *et al*., 1991; Cakmak *et al*., 1993; Aalen, 1999).

A potential role of TRX in reserve mobilization, proteolysis and redox status of storage proteins during germination has already been suggested (Lozano *et al*., 1996). Another general role is the protection against ROS that are highly produced with the resumption of metabolism (Leprince *et al*., 1990; Puntarulo *et al*., 1991; Cakmak *et al*., 1993; Aalen, 1999). TRX h has been suggested to act as hydrogen donor to 1Cys-peroxiredoxin, which protects embryo macromolecules from oxidation during early imbibition (Aalen, 1999). In connection with these observations, pea TRX h4 could play a role in sulphate assimilation and in the repair of protein damaged by oxidative stress (Mouaheb *et al*., 1998). TRX h4 expression is restricted to embryonic axes, where the assimilation of sulphate and the reduction of Met sulfoxide into Met could be crucial processes to provide sulphur containing amino acids that are necessary in embryo axes to sustain early protein synthesis. The function of TRX in vascular tissue was initially related to the differentiation of this tissue during the early stages of plant development (Ishiwatari *et al*., 2000).

CONCLUSIONS

Environmental pollution due to heavy metals is a major problem. It is a growing concern that the concentration of toxic metals including cadmium is rapidly increasing in soil due to several reasons such as mining, urban traffic, metal-working industries, mineral fertilizer, heating systems and waste incinerators. To elucidate how plants defend themselves from the toxicity of cadmium, extensive research has been conducted recently.

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