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The role of ferredoxin:thioredoxin reductase/thioredoxin m in seed germination and the connection between this system and copper ion toxicity

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ABSTRACT

Seed germination is highly sensitive to changes in the surrounding environment. This work examined the impact of imbibition with copper solution on the germination rate and behavior of some enzyme capacities involved in stress response. Chickpea (*Cicer arietinum* L.) seeds were germinated at 25 °C in the dark for 7 days of imbibition with distilled water or an aqueous solution of chloride salt of 100 or 500 μM $CuCl_2$. The exposure of seeds to copper (Cu^{2+}) induced changes in the antioxidant status. In Cu-treated seeds, the non-protein thiols (—SHNP) pool and ferredoxin:thioredoxin reductase (FTR) expression and activity increased. Cysteinyl sulfurs in the thioredoxin (Trx) function as ligands for metal ions. The accumulation of Cu^{2+} inhibited seed germination and embryo growth. It appears that the FTR system mediates a novel form of redox signaling in plants under copper excess.

Introduction

The ferredoxin:thioredoxin reductase/thioredoxin (FTR/Trx) system is involved in the regulation of enzymes, modulation of transcription factors or acts as a hydrogen donor and provides oxidative protection (Ford et al., 1987; Maheshwari et al., 1992; Dai et al., 2000; Manieri et al., 2003; Schürmann and Buchanan, 2008). Its function depends on the capacity to reduce disulfide bonds leading to activation or deactivation of the target. The roles include coupling of electrons and proton transfer in photosynthetic and respiratory electron transfer chains, substrate binding and activation, determination of protein structure, regulation of gene expression and enzymatic activity, iron, electron, and cluster storage, and disulfide reduction (Schürmann and Buchanan, 2008). Plants contain three Trx systems. The chloroplastic Trx system includes eight types of Trx (f, m, x, y, CDSP32, APR proteins, lilium proteins and HCF164) and a heterodimeric ferredoxin-dependent Trx reductase (Cain et al., 2009). The NADPH-dependent thioredoxin reductase (NTR)/thioredoxin (Trx) system catalyzes disulfide bond reduction in the cytoplasm and mitochondrion (Cazalis et al., 2006; Alkhalfioui et al., 2007). Shahpiri et al. (2008) suggest that different Trx isoforms are differentially regulated during germination. The production of reactive oxygen species by germinating seeds has often been regarded as a cause of stress that might affect the success of germination (Bailly et al., 2000). Therefore, antioxidant compounds and enzymes have been considered as being of particular importance for the completion of germination. The FTR/Trx is involved in light/dark regulation of chloroplast enzymes (Schürmann and Buchanan, 2008) and in oxidative stress responses (Balmer et al., 2003). The mechanisms regulating expression of Trx and FTR during the heterotrophic stage remain unclear.

The most common abiotic stressors affecting chickpea (*Cicer arietinum* L.) germination are drought, heat and cold, salinity, soil alkalinity and acidity, nutrient deficiencies and toxicities (Singh and Diwakar, 1994; Siddique et al., 1999; Ryan et al., 2008; Mbarek et al., 2013). Plants use a redundant combination of metal-regulated import inhibition, sequestration, and enhanced export mechanisms for protection against copper toxicity. Chickpea is an important food crop and is easily cultured and maintained in the laboratory. In previous work, we demonstrated that Cd causes oxidative stress through alterations in mitochondrial and cytosolic pea thioredoxins (Trx O and Trx h). The present work explains the role of chloroplast FTR:Trx m in seed germination and the connection between this system and metal ion toxicity.

Abbreviations: Cu, copper; FTR, ferredoxin:thioredoxin reductase; MDG, mean daily germination; —SHNP, non-protein thiols; Trx, thioredoxin.

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Materials and methods

Plant material and growing conditions

Seeds of var. Amdoun were disinfected with 2% sodium hypochlorite for 10 min. Seeds were germinated at 25 °C in the dark for 7 days over 2 sheets of filter paper moistened with distilled water or aqueous solutions of 100 or 500 μM $CuCl_2$. The germination rate was calculated as the percent of the control and germinating seeds sampled for assay. Mean daily germination was defined as the number of seeds germinating daily relative to the maximum number of germinated seeds. Germination speed was estimated by average time T_{50}; germination of 50% of seeds. At harvest, the seed coat was removed and the embryonic axes weighed and stored in liquid nitrogen until analysis, or dried for 8 days at 70 °C for dry weight determination.

Plant sampling

Embryonic axes were ground in a mortar with pestle in a medium (w/v = 1/5) containing 50 mM Tris–HCl (pH 8.0), 0.4 M sucrose, 1 mM EDTA, 5 mM ascorbic acid and 1 mM $MgCl_2$. The homogenate was squeezed through double cheesecloth and centrifuged at 5000 × *g* for 20 min. The supernatant obtained was carefully decanted and used for non-protein thiols, FTR and Trx assays. All procedures were carried out at about 4 °C.

Determination of non-protein thiols

Non-protein thiols were extracted by homogenizing the embryonic axes supernatant in 5% (w/v) sulfosalicylate (pH < 1), containing 6.3 mM diethylene triamine pentaacetic acid. After centrifugation at 10,000 × *g* for 30 min at 4 °C, supernatants were used for analysis and —SHNP determined in homogenates spectrophotometrically at 412 nm using 5,5′dithio-bis-(2-nitrobenzoic acid) (Anderson, 1985).

Determination of copper contents

After wet digestion, concentrations of Cu were determined by an atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA) using Sigma Diagnostic Standard.

Enzyme assays

Trx m activity was determined according to Wolosiuk et al. (1979). NADP–malate dehydrogenase (MDH) was pre-incubated at 25 °C in 0.1 mL of a solution containing Trx m extract, 10 μmol of Tris–HCl buffer (pH 7.9), and 1 μmol of dithiothreitol. After preincubation for 10 min, the mixture was injected into a 1 cm cuvette of 1 mL capacity containing 100 μM Tris–HCl buffer (pH 7.9) and 0.25 μM NADPH. The reaction was started by addition of 2.5 μmol oxalacetic acid. The change in absorbance at 340 nm was measured with a spectrophotometer (model 500, Cary, Rockland, MA).

FTR (EC 1.8.7.2) activity was measured spectrophotometrically with NADPH oxidation at 340 nm in a coupled system. The reaction was carried out at 25 °C in 500 μL reaction mixture containing 30 mM Tris–HCl, pH 8.0, 200 μM NADPH, 5 μM Fdx, 10 μM Trx m and enzyme extract.

Control assays were carried out without substrates or extracts. One unit of enzyme was defined as the amount necessary to decompose (or produce) 1 μmol of substrate (or product) per min at 25 °C.

Table 1
Germination rate of *Cicer arietinum* seeds after imbibition with H_2O or 100 or 500 μM $CuCl_2$ at 7 days after sowing.

Treatment × time		Germination %	MDG[a]	T_{50}
Control	0	0		
	1	4 ± 2		
	3	67 ± 5		
	5	85 ± 6		
	7	96 ± 4	14	3
Cu (100 μM)	0	0		
	1	2 ± 1		
	3	25 ± 3		
	5	48 ± 5		
	7	69 ± 3	10	5
Cu (500 μM)	0	0		
	1	2 ± 1		
	3	21 ± 3		
	5	29 ± 4		
	7	46 ± 5	6	7

[a] MDG, mean daily germination; T_{50}, germination speed of 50% of seeds. Data mean of two independent experiments, each of which comprised of 80 seeds. Differences were considered significant at $P < 0.05$.

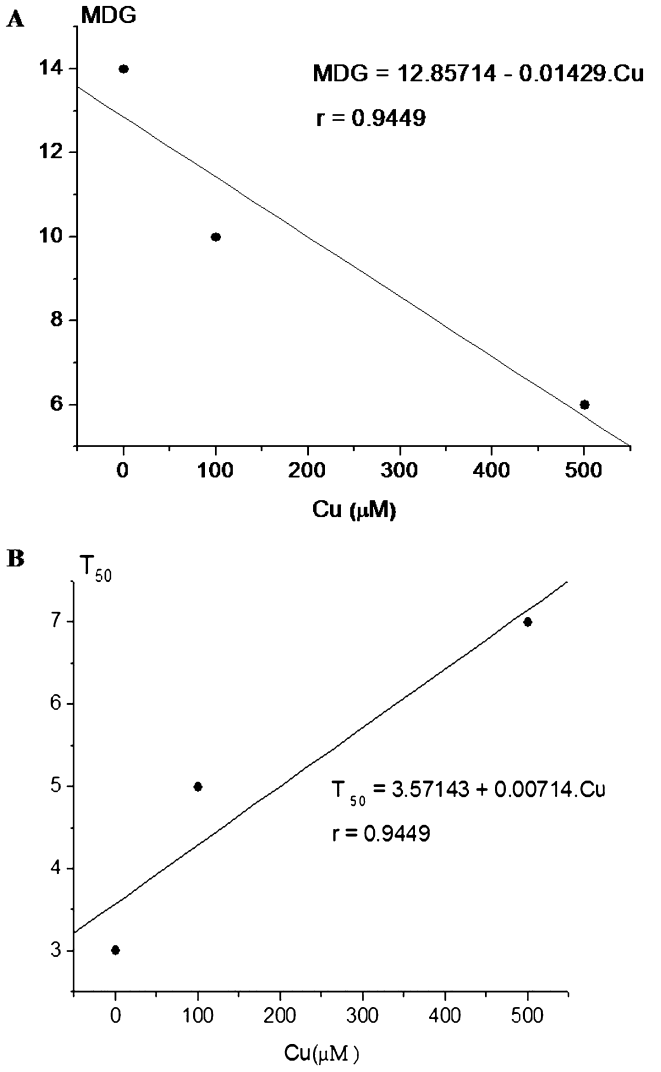

Fig. 1. The mean daily germination (MDG) and germination speed of 50% of seeds (T_{50}) were analyzed with least significant difference.