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High specificity in response of pea mutant SGECd^{t} to toxic metals: Growth and element composition

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ABSTRACT

The present report aimed to better understand the mechanisms of plant co-tolerance to various toxic metals, and relationships between metal tolerance and metal accumulation. The pea (*Pisum sativum* L.) line SGE and its mutant SGECd^{t}, having increased tolerance to and accumulation of Cd, but decreased tolerance to and accumulation of Hg, were cultivated in hydroponics at a range of toxic concentrations of heavy metals (Cd, Co, Cr, Hg, La, Ni, Pb and Zn), as well as Al, Fe, Mn, NaCl and H^{+} ions. The SGECd^{t} mutant showed increased tolerance to Co (increased root biomass at 12 and 25 μM Co and shoot biomass at 25, 50 and 100 μM Co), but similar root and shoot Co contents as SGE. No significant differences between SGE and SGECd^{t} in biomass response to other metals and low pH were detected. However at particular metal concentrations, SGECd^{t} tended to (Student's *t* test, $P < 0.05$) have increased: (i) shoot biomass (34%) in the presence of 400 μM Zn; (ii) root and shoot biomass (32%) in the presence of 100 μM Fe; (iii) root Mn or Zn contents (65% or 8%, respectively) in the presence 400 μM Mn or Zn, compared to SGE plants. No genotypic differences in the content of other toxic metals were observed, except for the previously reported increased Cd content and decreased Hg content in SGECd^{t}. Generally, metal toxicity decreased macro- and micro-element (nutrient) concentrations in plants, however opposite effects were also observed particularly on Hg-treated plants. SGECd^{t} had increased root Ca, Fe, Mg, Mn and S content and shoot B, Ca, Mg, Mn, Na and Zn content in Cd-treated plants. In the presence of toxic Hg the mutant contained less root and shoot Ca, K, Mg and S, but had increased root Co, Cr and Cu contents. Genotypic differences in individual nutrient elements were also observed following Ag, Al, La, Mn, Ni or Zn treatment. Taken together, the results indicate high specificity in phenotypic responses of SGECd^{t} exposed to toxic metals and that the mutation might affect some regulatory genes, which could modulate nutrient (particularly Ca) homeostasis and regulation of ion transporters.

1. Introduction

Heavy metals are widespread soil pollutants that can inhibit plant growth and accumulate in agricultural products. A number of elements such as Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn belong to the group of heavy metals, and physical and chemical properties of these elements vary greatly (Housecroft and Sharpe, 2008). Plants developed several mechanisms for decreasing or avoiding toxicity of heavy metals and preventing their excessive uptake by roots and translocation to reproductive organs. Although some interactions between plants and toxic metals are common for all or most heavy metals, some mechanisms are specific or prevalent for a particular metal (Patra and Sharma, 2000; Hall, 2002; Clemens, 2006; Dong et al., 2007; Nagajyoti et al., 2010; Verkleij et al., 2009; Lin and Aarts, 2012). Usually reports on this topic are devoted to a single metal, and most usually Cd (summarized by Sanita di Toppi and Gabrielli, 1999; Dong et al., 2007; Hasan et al., 2009), thereby missing important information about responses of the studied plant species or genotypes to other elements.

Mutagenesis is a powerful tool to understand the mechanisms of accumulation of, and tolerance to, toxic metals by plants. The important role of phytochelatins in Cd detoxification was shown using Cd-sensitive mutants *cad1* and *cad2* of *Arabidopsis thaliana* deficient in phytochelatin synthase (Howden et al., 1995) and γ-glutamylcysteine synthetase (Cobbett et al., 1998), respectively.

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Decreased P, K, Mg, S and Fe concentrations in the Cd-treated *cad1* mutant suggested that maintaining nutrient homeostasis contributes to Cd tolerance (Larsson et al., 2002). Experiments with a Cd-sensitive mutant of rice demonstrated that translocation of Cd from root to shoot may be mediated by Zn and Mn transporters exacerbating Cd toxicity in shoot (He et al., 2009). The Cd-tolerant *A. thaliana* mutant MRC-32 contained more Cd but exhibited pleiotropic phenotype with slow growth rate and alterations in leaf development, indicating that the mutation occurred at a regulatory gene controlling expression of several other genes (Watanabe et al., 2010). Another *A. thaliana* mutant MRC-22 had a Cd-phobic root response, suggesting mis-regulation of Cd sensing in the root zone (Watanabe et al., 2010). The *A. thaliana* mutant *cup1-1* with increased sensitivity and accumulation of Cd and Cu was similar to WT in its response to Hg, suggesting that different, metal-specific mechanisms were involved (Van Vliet et al., 1995).

Several mutants having altered metal relations were isolated and described in pea. Two pea mutants A79-397 (Gottschalk, 1987) and E107 (Welch and LaRue, 1990) were characterized by elevated exudation rate of Fe(III)-reducing substances to the surrounding medium resulting in increased sensitivity to Fe toxicity, which excessively enhanced Fe uptake and caused necrotic spots on the leaves. In addition, the E107 mutant excessively accumulated aluminum and manifested symptoms typical of Al toxicity (Guinel and LaRue, 1993). A chemically induced mutant SGECd^t was isolated from a laboratory pea line SGE and characterized by increased Cd tolerance and Cd accumulation (Tsyganov et al., 2007). In the presence of toxic Cd (4 μM), the mutant SGECd^t had lower contents of non-protein thiols and free proline, lower activities of catalase and peroxidase than wild-type (WT) plants, and maintained plant nutrient uptake, suggesting a Cd-insensitive phenotype. Under optimal conditions the SGECd^t mutant had 35% more oxidized glutathione in the roots than SGE plants. However it was concluded that mutation was not linked to glutathione and/or phytochelatin biosynthesis, since only marginal genotypic differences in their concentrations were found in Cd-treated plants (Tsyganov et al., 2007). A crucial role of the root in the increased Cd-tolerance and Cd-accumulation of SGECd^t was shown using reciprocally grafted plants (Malkov et al., 2007). SGECd^t had better water uptake by Cd-treated plants and higher root sap flow rate in both the presence or absence of toxic Cd (Belimov et al., 2015), suggesting that root water transport might be involved in mechanisms of increased tolerance and accumulation of Cd (Belimov et al., 2015). In contrast, Hg treatment of SGECd^t revealed decreased Hg-tolerance and foliar Hg-accumulation but had more negative effects on plant water relations compared to SGE plants (Belimov et al., 2015).

The present report aimed to characterize genotypic specificity of SGECd^t mutant in tolerance to and uptake of different heavy metals, as well as Al, Fe, Mn, NaCl and H^+ ions, to better understand mechanisms of metal co-tolerance, relationships between metal tolerance and accumulation, and effects of toxic metals on plant nutrient uptake.

2. Materials and methods

2.1. Plant growth conditions

The wild-type pea (*Pisum sativum* L.) line SGE and its Cd-tolerant mutant SGECd^t (see Introduction section for details) were used. Seeds were surface sterilized and scarified by treatment with 98% H_2SO_4 for 30 min, rinsed carefully with tap water and

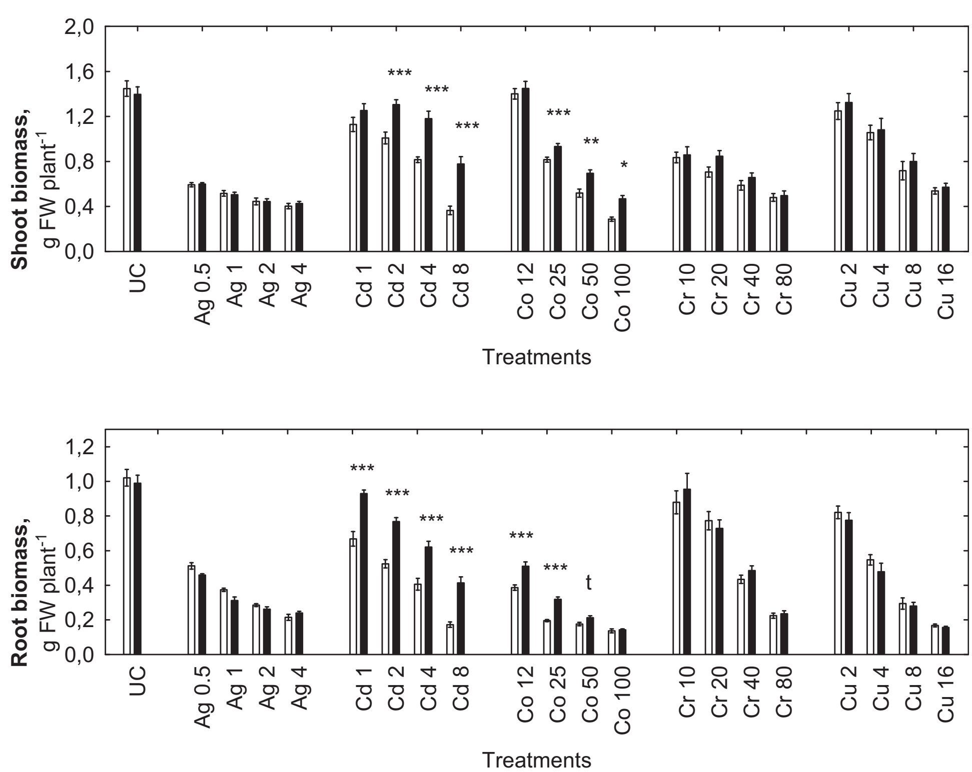

Fig. 1. Growth response of pea plants to toxic concentrations of Ag, Cd, Co, Cr and Cu.
Pea genotypes: wild type SGE (□), mutant SGECdt (■).
The axis of abscissa listed elements and their concentration in μM.
UC stands for untreated control in the complete nutrient solution
Asterisks show significant difference between pea genotypes at P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***) as determined by Fisher's LSD test (two way ANOVA; n ≥ 10).
t shows significant difference between pea genotypes at P < 0.05 as determined by Student's test (n = 15) for a given treatment.