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Cadmium effects on embryo growth of pea seeds during germination: Investigation of the mechanisms of interference of the heavy metal with protein mobilization-related factors

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ABSTRACT

This work aims to give more insight into mechanisms of action of cadmium (Cd) on germinating pea seeds (*Pisum sativum* L. var. douce province), specifically the different ways by which Cd cations may interfere with the principal factors involved during germination process, notably storage proteins mobilization, amino acids freeing and proteolytic activities. Obtained results revealed that the process of hydrolysis of main storage proteins showed a significant disruption, which resulted in the decrease of the release of free amino acids, thus imposing a lack in nitrogen supply of essential nutrients to growing embryo under Cd stress. This hypothesis was evidenced by Cd-induced changes occurring in main purified protein fractions; Albumins, Legumins and Vicilins, during their breakdown. Besides, at enzymatic level, the activities of main proteases responsible for this hydrolysis were altered. Indeed, assays using synthetic substrates and specific protease inhibitors followed by protease activity measurements demonstrated that Cd inhibited drastically the total azocaseinolytic activity (ACA) and activities of different proteolytic classes: cysteine-, aspartic-, serine- and metallo-endopeptidases (EP), leucine- and proline-aminopeptidases (LAP and PAP, respectively), and glycine-carboxypeptidases (Gly-CP). The data here presented may suggest that the vulnerability of the embryonic axes towards Cd toxicity could be explained as a result of eventual disruption of metabolic pathways that affect mobilization of reserves and availability of nutrients. In *vitro* studies suggest that Cd cations may act either directly on the catalytic sites of the proteolytic enzymes, which may cause their deactivation, or indirectly via the generation of oxidative stress and overproduction of free radicals that can interact with enzymes, by altering their activity and structure.

1. Introduction

Legume seeds, in particular pea seeds (*Pisum sativum* L.), represent great nutritional importance due to their high-quality source of protein and starch (Hedley, 2001). Research interests have focused on physiological, biochemical and molecular processes relevant for agriculture, including the mobilization of reserve proteins during seed germination, concomitant with the allocation of nitrogen resources (free amino acids) to seedling (Schaller, 2004). In developing seeds, storage proteins were deposited within storage organs, particularly accumulated in protein bodies. They consist, mainly, in albumins (around 30% of total proteins) and globulins (around 50% of total proteins), which are soluble, respectively, in water and salt solution (Schroeder, 1982). Albumins were characterized into two dimers PA1 (PA1a; 6 kDa and PA1b; 4 kDa), PA2 (26 kDa) and tetramer lectin (50 kDa), while globulins were classified by their sedimentation coefficients into convicilin (7S fraction, trimer of 70 kDa), vicilin (7S fraction, trimer of 71 kDa) and legumin (11S fraction, 380–410 kDa) with 6 subunits (α acid of 40 kDa and β basic of 20 kDa, which are linked by a disulfide bridge) (Gatehouse et al., 1984).

At the onset of seed germination, mobilization of storage proteins may start in sink tissues within the local embryonic reserves; pending the mobilization of cotyledonary ones (Tiedemann et al., 2000; Muccifora et al., 2010). Typically, this process involves various

Abbreviations: AP, aminopeptidase; CP, carboxypeptidase; EP, endopeptidases; E-64, L-*trans*-epoxysuccinyl-leucylamide-4-guanidino-butane; FW, fresh weight; LAP, leucine aminopeptidases; MW, molecular weight; PAP, proline aminopeptidases; PMSF, phenylmethylsulfonylfluoride; Pro-ßNA, L-proline-ß-naphthylamide

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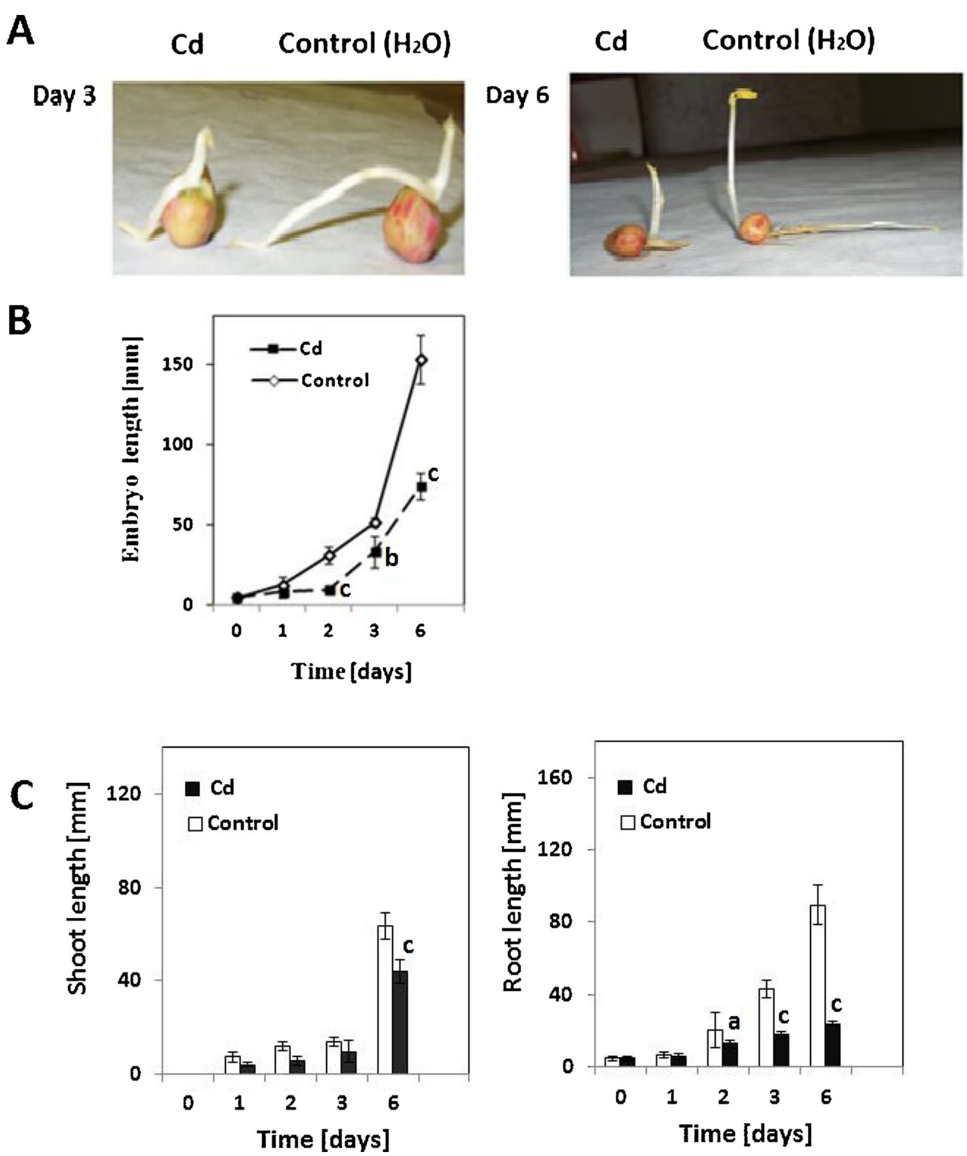

Fig. 1. Germination of pea (*Pisum sativum* L. var. douce province) seeds for 3 and 6 days in the presence of H_2O (Control) or 200 µM $CdCl_2$ **(A)**. Embryo growth evaluated as the whole embryonic axis length in control and Cd germinated seeds **(B)**. Length of shoot and root of embryos **(C)**. Significant differences of controls to Cd were designed according to p values by "**a**": $p < 0.05$, "**b**": $p < 0.01$ and "**c**": $p < 0.001$.

proteases with specific cleavage sites; on the interior of peptide chains (endopeptidases, EP) or on termini (exopeptidases; aminopeptidases, AP and carboxypeptidases, CP) (Huffaker, 1990). Proteases can also be classified according to their catalytic mechanism into four classes; serine (EC.3.4.21), cysteine (EC.3.4.22), aspartic (EC.3.4.23) and metallo- (EC.3.4.24) proteases (Powers et al., 2002).

Because of their sessile character, plants must confront diverse environmental stresses during their life. Currently, with rapid industrial development, heavy metals represent a serious problem in agricultural production. Seed germination and subsequent embryo growth are important stages of plant life and highly sensitive to surrounding medium fluctuations, because germinating seed is the first interface of material exchange between plant development cycle and environment. Several studies have focused on impact of abiotic and biotic environmental stresses on seed germination and plantlet growth (El-Jaoual and Cox, 2008; Rahoui et al., 2010; Jaouani et al., 2012; Akhzari et al., 2016; Mozafariyan et al., 2017).

Cadmium (Cd) is an environmental pollutant extremely toxic to plants and other living organisms including humans (Mendiola et al., 2011). Contamination by heavy metals, in particular Cd, has been shown to cause many physiological and metabolic alterations in germinating seed and growing seedling; e.g., respiratory disturbances (Smiri et al., 2010), limitation in the availability of nutrients (minerals and carbohydrates) (Rahoui et al., 2010; Sfaxi-Bousbih et al., 2010a,b,c) and dysfunction in the photosynthesis (Baszyński, 2014; Almansa et al., 2016).

Hence, many studies were carried out to characterize the toxicity of heavy metals on plant crops, seeds germination and seedlings development (Vázquez de Aldana et al., 2014; Cokkizgin and Cokkizgin, 2015; Han et al., 2015; Prodanovic et al., 2016). In this concept, the present work may highlight informational gaps relating to understanding exact mechanisms by which Cd cations may interfere at molecular levels in germinating seeds. Therefore, the effects of Cd on embryos and cotyledons of pea seeds were assessed with respect to protease activity, kinetic breakdown of storage proteins, as well as the release of ultimate products of their hydrolysis (free amino acids).