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HSP70 LEVEL IN THE LEAVES OF *PHASEOLUS VULGARIS* INTOXICATED WITH CADMIUM.

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The heavy metal cadmium has been recognized as an extremely hazardous environmental contaminant that can be highly phytotoxic. Excessive amounts of cadmium in the environment can affect the performance of plants at various levels of biological organization, from the cellular up to the ecosystem level. Exposure to this harmful heavy metal can cause disturbance of several essential physiological processes in plants, including photosynthesis, respiration and transpiration (Seregin and Ivanov, 2001; Ciscato et al., 2002). At the cellular level, toxic effects of cadmium may result from binding of the metal to sulfhydryl groups of proteins, leading to disruption of protein structure and function (Hall, 2002).

Plants have a range of potential protective mechanisms at the cellular level that are involved in detoxification and thus in development of tolerance to heavy metal-induced stress. The cellular stress response is a general defense mechanism commonly seen in all organisms, from bacteria to higher organisms, including plants. This response is characterized by rapid and transient synthesis of heat shock proteins (HSPs) (Nover, 1991; Sanita di Toppi and Gabbrielli, 1999). Appearance of damaged proteins in the cell after cadmium treatment serves as a signal that triggers the activation of HSP genes, whose products (HSPs) prevent and repair protein damaged (Hightower et al., 1994). As a member of the HSP family, HSP70 plays an important role under stressful conditions, serving to minimize injury and assist in cellular recovery (Pelham, 1984). Several different HSP70 isoforms have been described in plants; some of them are predominantly constitutively expressed, while others are inducible. There are only a few reports indicating that cadmium intoxication provoked induction of HSP70 in kidney bean (Leita et al., 1991, 1993).

The kidney bean (*Phaseolus vulgaris* L.) is a wide spread and important edible agriculture legume. Accumulation of cadmium in kidney bean can lead to a decrease in the yield of this plant and provoke hazardous effects on human health through the food chain. Also, kidney bean is easy to grow and has a short generation time, which facilitates research on this plant.

In the present study, the expression of HSP70 in bean seedlings exposed for 5 or 15 days to increasing doses of cadmium was examined in order to learn more about the role of HSP70 in the ability of *P. vulgaris* to cope with chemical stress provoked by the toxic metal cadmium.

Selected healthy beans were grown 20 days on plates with cotton soaked in Knop's solution as a nutrient medium under optimal light and temperature conditions. Over the next 5 or 15 days, the formed seedlings were further watered with increasing doses of cadmium (1 μM - 100 μM Cd). Treatment was performed two times per day with 4 ml of $CdCl_2$ solution. Untreated plants were watered with the same volume of Knop's solution. After the treatments, whole cell extracts were prepared from collected leaves by pulverization and sonification in 0.1 M Tris buffer, pH 7.6, containing 1 mM dithiothreitol and 1 mM EDTA. Proteins (50 μg) from the leaf cellular extracts were resolved on 7.5 % SDS PAGE at 4°C according to Laemmli (1970). Western transfer of proteins from the gel to the PVDF membrane was performed at 135 mA overnight in transfer buffer. Both constitutive and inducible HSP70 isoforms were detected by the N27F3-4 anti-HSP70 antibody followed by the alkaline phosphatase-conjugated counter antibody. Immunoreactive proteins were visualized by an enzyme-amplified chemifluorescence (ECF) method. Quantitative analysis of immunoreactive bands was performed using ImageQuant software.

In order to examine the HSP70 level in leaf extracts of bean seedlings treated for 5 or 15 days with increasing doses of cadmium, a quantitative Western blot procedure was performed. The results presented on Fig. 1 show that 5-day treatment of bean seedlings with 1 μM, 10 μM, and 25 μM Cd, regarded as low or moderate doses (Mishra et al., 2006), increased the HSP70 level by about 60% above its level in untreated plants. Under such conditions, kidney bean cells respond in the way expected and well documented in various organisms. Application of higher cadmium doses (50 μM and 100 μM Cd) over a 5-day period did not affect the HSP70 level. Interestingly, after prolonged treatment (15 days) with low doses of cadmium (1 μM, 10 μM Cd) the HSP70 level in bean seedlings also remained unaltered (Fig. 1). Assuming that the plants treated for 15 days with low doses of cadmium and plants treated for 5 days with high doses of the metal accumulate comparable amounts of cadmium, the unchanged HSP70 level could be attributable to activation of other cellular protective mechanisms, such as induction of other members of the HSP family and/or phytochelatines.

An additional factor that should be taken into a consideration in attempting to explain the unchanged HSP70 level observed in plants treated for 15 days with low doses of cadmium

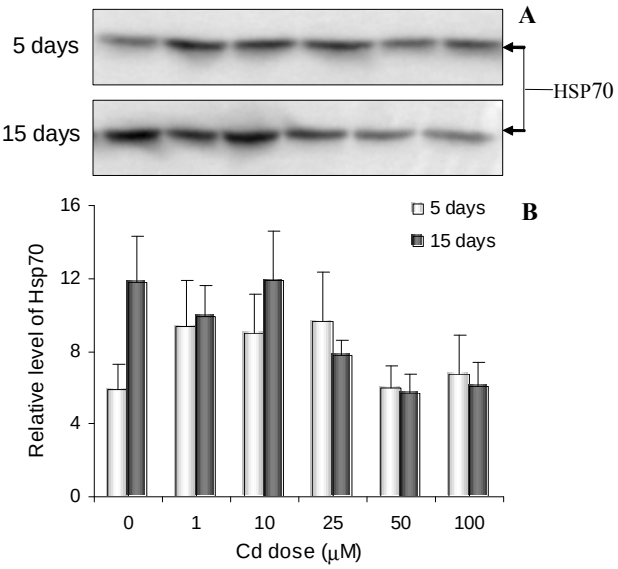

Fig. 1. HSP70 level in leaves of *P. vulgaris* intoxicated with cadmium. (A) Leaf cell extracts were subjected to SDS-PAGE and Western blotting. HSP70 was detected by the N27F3-4 antibody followed by the counter antibody and ECF reaction. The representative blots are shown. (B) Data obtained after quantification of immunoreactive bands by ImageQuant software.

is the basal level of constitutively expressed Hsp70. Plant HSP70 isoforms are encoded by a multi-gene family whose members are developmentally regulated and differentially expressed in response to various physiological and stressful conditions, including heavy metal intoxication. It was previously established that 12 genes encode HSP70 proteins in spinach and even 14 genes in *Arabidopsis* (Guy and Li, 1998; Sung et al., 2001). At least three of them are constitutively expressed (Sung et al., 2001). These HSP70 isoforms have important functions in plant growth and development, as well as in the plant response to environmental stress (Guy and Li, 1998; Sung et al., 2001; Chen et al., 2004). Assuming that plants watered solely with Knop's solution express only constitutive HSP70 isoform, it was of interest to compare the basal levels of this isoform in leaf extracts of untreated plants. The results presented on Fig. 1 show that seedlings watered for an additional 15 days with Knop's solution have a two-fold greater quantity of constitutive HSP70 than in seedlings watered for an additional 5 days. The amount of constitutively expressed HSP70 in plants receiving 15-day treatment seems to be sufficient to provide protection against toxic effects of low doses of cadmium, although the possible involvement of other protective mechanisms cannot be excluded.

Finally, application of a moderate cadmium dose (25 μM Cd) for 15 days decreased the HSP70 level by approximately 30%, while higher doses (50 μM and 100 μM Cd) reduced the HSP70 level by 50% in comparison with untreated plants (Fig. 1). It is possible that during 15-day treatment with moderate and high doses, plants accumulate cytotoxic amounts of the metal leading to reduction of the HSP70 level. This result suggests that the adverse effects of cadmium apparently overcome the protective capacities of HSP70 and other detoxification mechanisms.

In conclusion, the level of HSP70 in kidney bean leaves depends on the amount of applied and accumulated cadmium, as well as on duration of the treatment. The basal level of constitutive HSP70 in untreated plants increased during growth. On the whole, the presented data show that both constitutive and inducible HSP70 isoforms play an important role in the respons to stress provoked by cadmium and imply the involvement of other protective mechanisms. Our further studies along these lines will be focused on synthesis and tissue distribution of other Hsps and phytohelatines.

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