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Cadmium tolerance and accumulation characteristics of mature flax, cv. Hermes: Contribution of the basal stem compared to the root

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HIGHLIGHTS

- Cd accumulated in stem bottom part exceeded the defined hyperaccumulator threshold.
- No toxic symptoms occurred and TI of all growth parameters ranged between 0.7 and 1.
- The high level of Zn, Mn and Cu may contribute to the absence of chlorosis in stem.
- Cd/Ca synergistic effect observed in the stem may alleviate Cd toxicity.
- Hermes variety accumulated more Cd than the other flax varieties ever described.

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ABSTRACT

The potential of mature flax plants (cv. Hermes) to tolerate and accumulate cadmium (Cd) was studied to determine which part of the plant would be the key organ for phytoremediation purposes. After 4 month-growth on sand substrate containing 0.1 mM Cd in a greenhouse, the roots and stems were separated and the stems were divided into three parts. The effects of Cd were studied on growth parameters, histology and mineral nutrition. No visible toxic symptoms were observed. Tolerance-index values calculated from growth parameters and nutrients remained relatively high, allowing the development of the plant until maturity and formation of seeds. The roots and bottom stem accumulated the highest quantity of Cd (750 and 360 mg/kg dry matter), values which largely exceeded the threshold defined for hyperaccumulators. On the other hand, basal stem had a high bioconcentration factor (BCF = 32) and translocation factor TF′ (2.5) but a low TF (0.5), indicating that this basal part would play a major role in phytoremediation (phytostabilization rather than phytorextraction). Therefore, the high tolerance to Cd and accumulation capacity make possible to grow Hermes flax on Cd-polluted soils.

1. Introduction

Cadmium (Cd) is one of the most hazardous and ubiquitous trace elements present in the soil that is toxic for all biological systems [1]. Its level has been increased in the environment due to the anthropogenic activities, including the expansion of the industry and the use of some agricultural practices: waste water irrigation, application of sewage sludges and the excessive use of fertilizers and pesticides. The consequence is that forests and a number of cultivated lands cannot be exploited leading to a decrease of biodiversity. In order to reduce the Cd pollution, the tolerance and accumulation properties of many plants have been studied. When usual species accumulate 0.05–0.2 mg of Cd/kg of their dry leaves, hyperaccumulator plants have the capacity to concentrate more than 100 mg/kg of dry matter (DM) in harvested organs [2]. Only a few species are known to hyperaccumulate Cd, including *Thlaspi caerulescens* [3] and *Arabidopsis halleri* [4]. These hyperaccumulators are slow growing and produce a low biomass. On the other hand, crop species such as tobacco, flax, cotton, maize, sunflower, or fast growing trees (poplar, willow), with phytoextraction potential (10–100 mg/kg Cd MS^{-1}) and high biomass production, have been introduced in the phytoremediation concept to overcome the mass limitation of hyperaccumulators [5,6]. The possibility of using flax (*Linum usitatissimum*) in phytoremediation strategy has been reported by several authors [*e.g.* 7,8]. More particularly, the accumulation of Cd in flax was compared in different cultivars to search for genotype differences in metal uptake and distribution in the

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various organs of the plant, mature or taken at different stages of growth [9]. Other studies have compared the Cd accumulation and tolerance capacity of flax with other crops such as cotton and hemp [10,11].

Angelova et al. [10] showed that in mature flax (cv. Kaliakra) growing in an industrially polluted region with Cd concentration reaching 12 mg/kg soil, the amounts of Cd accumulated in roots (8.7 mg/kg DM) and stems (7.3 mg/kg DM) were comparable and much higher than in leaves, capsules and seeds (≤2 mg/kg DM). They also found that flax absorbed and accumulated more Cd than cotton and hemp. Studying six fibre-flax and four linseed cultivars (grown in soil supplemented with 10–1000 mg/kg Cd), Bjelková et al. [9] found that (1) most Cd was accumulated in roots (reaching 60–80 mg/kg DM at 1000 mg/kg) being 2–8 times higher than in other organs, and (2) fibre flax accumulated more Cd than linseed. Working on 28-day old flax (cv. Longya7) cultivated in the presence of Cd concentrations varying between 50 and 200 mg/kg soil, Shi and Cai [11] found that (1) Cd content in the shoots was >100 mg/kg, and (2) flax, with hemp and peanut, were more tolerant and have higher Cd accumulation capacity compared with sunflower and soybean. Although the Cd accumulated in flax was often lower than the values reported for hyperaccumulators, all these authors concluded that flax might be an excellent candidate for phytoremediation.

Our previous data on flax seedlings (cv. Hermes) have shown a high accumulation of Cd in roots (up to 3000 mg/kg DM) as well as in aerial parts (670 mg/kg DM). Nevertheless, these data have been collected after 10–18 days-culture in hydroponic conditions [12,13]. The objective of the present research was to investigate the impact of Cd on the same variety but grown on sand moderately polluted by Cd (11 mg/kg dry soil) and harvested at maturity, in comparable conditions as those reported by Angelova et al. [10]. We studied the adaptative response to Cd and tolerance characteristics of the plants, looking at the impact of Cd on growth, stem anatomy and nutrient uptake. Moreover, the Cd accumulation was investigated at different levels of the stem axis in comparison with the whole stem and roots. Our data point out, for the first time, that the basal segment of the stem is a key part for Cd accumulation.

2. Materials and methods

2.1. Plants, materials and growth conditions

Seeds of flax (*L. usitatissimum*, cv. Hermes), a gift of the Cooperative Terre de Lin, France) were sown into pots (25 cm × 75 cm) filled with commercial grade sand. The plants were grown in a green-house (average T = 20–25 °C; photo-period 16 h light/8 h dark) and were irrigated regularly with a culture medium [additional Table] supplemented with either 2.6 mM $Ca(NO_3)_2$ for control or 0.1 mM $Cd(NO_3)_2$ and 2.5 mM $Ca(NO_3)_2$. Plants were harvested after four month growth.

2.2. Growth analysis

The root and stem were separated. The stem length (until the inflorescence; n = 30) and radius (in the middle of the stem; n = 10 among 30 stems with values closest to the average length) were measured. The leaves number and internode distance on the stem were also determined (n = 10). The stem (4–5 g) was divided into three equal-length parts (top, middle and bottom). External tissues enriched in fibres (ETFs) were mechanically separated from shives and stored at 40° overnight and then at 80 °C (2 h × 1 h) to determine the weight of dry matter. The weight of 100 seeds was also determined.

2.3. Histological study

After alcohol dehydratation, the middle of the stem was subjected to hand transverse cross-sections and staining with carmine-green as previously described [14]. Sections were observed with a light microscope (Leica microsystem, Austria). An anatomical study was performed, firstly to check the general tissue organization. Secondly, the fibre-bundle (B) number, the number of fibres (F) per bundle, the total fibre number were counted; the fibre diameter and the thickness of fibre wall were determined. Ten representative stems were used for both treated and non-treated plants and several sections (n = 15) were observed from which the best one was selected for light microscopy photographs and image analyses (using Paint Shop Pro software).

2.4. Determination of the plant content of cadmium and other cations

After digestion of dried samples (250–500 mg) in $HNO_3/HClO_4$ (3/1, v/v), the residue was diluted in 20 ml HNO_3 (1%, v/v) and the content of Cd and cations (Ca, Mg, Na, K, Mn, Fe, Zn, Cu) in the organs and seeds as well as in ETF and shives of the different parts of the stem was measured by atomic absorption spectrometry (Analyst 300, Perkin-Elmer, USA).

Average values of cation concentration were calculated from 3 measurements and 3 repetitions with less than 10% standard error.

2.5. Data analysis

The tolerance index (TI) measures the ability of the plant to grow in the presence of a given concentration of metal. Following Wilkins [15], most authors calculated TI as the ratio between the mass or length of an organ subjected to metal treatment compared with the control ones (*e.g.* [16]). In our case, TI was calculated on the basis of growth parameters including: number, diameter and morphology of fibres, yield of seeds, leave number, stem length and radius, xylem area. TI was also calculated for the nutrient accumulation. TI = [growth parameters] Cd/[growth parameters] control.

The translocation factor (TF) measures the ability of the plant to translocate the metal from the roots to the shoots [17]. TF was calculated using Cd concentration (mg Cd per kg DM) as TF = [Cd] stem tissue/[Cd] roots. On the other hand, TF′ was calculated using Cd amount (mg) accumulated in the organ (designated as Cd accumulation); thus TF′ = Cd accumulation in stem/Cd accumulation in root.

The bioconcentration factor (BCF) evaluates metal accumulation efficiency, and was calculated as in Brooks [2]: BCF = [Cd]plant tissue/[Cd]medium.

3. Results

3.1. Effect of cadmium treatment on plant morphology and growth

Under Cd treatment, flax plant grew steadily until maturity; chlorosis and necrosis were not visually observed. However, several side effects of the treatment were observed. The stem/root ratio (r), calculated from the biomass of dry organs, decreased in the presence of Cd (r = 14) compared with control (r = 17). A significant decrease affected the stem length and radius of about 34% and 24%, respectively compared with control (Table 1). Likewise, the average internode-distance was reduced from 4.0 ± 0.3 to 3.3 ± 0.3 mm. Such distances corresponded to the growth rate of the stem per day and remained in the same range of those reported during the fast period of growth of plants cultivated in the field [18,19]. This points out the optimal condition of growth in the green-house. On