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Transcriptome profiling of genes differentially modulated by sulfur and chromium identifies potential targets for phytoremediation and reveals a complex S–Cr interplay on sulfate transport regulation in *B. juncea*

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HIGHLIGHTS

- A coordinated regulation of sulfate transporters may occur in response to Cr.
- Cysteine is involved in the short-time response of plants to Cr exposure.
- Potential gene targets for Cr phytoremediation have been identified.

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ABSTRACT

A differential display cDNA–AFLP derived technique was used to identify gene transcripts regulated by chromium (Cr) in relation to sulfur (S) nutrition in *Brassica juncea*. Twelve-day old plants were grown with 200 μM sulfate (+S), without sulfate (−S), with 200 μM sulfate plus 200 μM chromate (+S + Cr), or without sulfate plus 200 μM chromate (−S + Cr). Forty-four combinations of degenerate primers were assayed, which allowed the detection of 346 Transcript-Derived Fragments (TDFs) differentially regulated by Cr and S at times 0, 10 min, 1 h, 24 h. Eight sulfate transporters were identified, whose transcript abundance was dependent on the levels of plant S-compounds. For some of these transporters, a tight coordinated regulation of gene expression was observed in response to Cr. The MapMan analysis revealed a differential pattern of gene expression between +S + Cr and −S + Cr plants for several other transcripts and highlighted an overlap among responses to metals, defence against pathogens and senescence, hence suggesting the existence of common mechanisms of gene regulation. Among the identified gene transcripts, those involved in S metabolism and proteolitic processes may represent potential targets of genetic engineering in efforts to increase Cr accumulation and tolerance in plant species employed in phytoremediation techniques.

1. Introduction

Among heavy metals, chromium (Cr) is regarded as one the most harmful inorganic pollutants that pose a risk to human health [1]. In nature Cr exists in two main stable oxidation states, the trivalent (+3) and the hexavalent (+6) [2]. These forms can interchange depending on physical, chemical and biological processes occurring in waters and soils, and greatly differ in toxicity [3]. Cr(VI) compounds (chromates and dichromates), in particular, are extremely mobile in the environment and display strong oxidizing and carcinogenic properties [4,5].

To date, a role for Cr in plant metabolism has not yet been recognized. Therefore, higher plants likely do not possess specific mechanisms to acquire this element [6]. Studies concerning the uptake of Cr from soil by different plant species have shown that Cr(III) is taken up passively, while Cr(VI) transport over plasma membranes is active and seems to be mediated by transporters of essential anions, such as sulfate [7–13].

The competition between sulfate and chromate for transport into cells has been recently investigated. In maize, chromate concentrations ranging within 0.05–1 mM specifically inhibited sulfate influx over a short-time period, and two days of 200 μM chromate exposure down-regulated the maize root high affinity

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sulfate transporter *ZmST1;1* [12]. Similar results were also obtained in 7-day old *Brassica juncea* seedlings treated for 48 h with Cr 1 mM, where the reduction in sulfate uptake capacity and decreased gene expression of the low affinity sulfate transporter *BjSultr2;1* were observed, concomitantly with the enhancement of cysteine and glutathione biosynthesis [13].

The involvement of sulfate transporters in chromate uptake is also supported by investigation on transgenic plants. The constitutive expression of the SHST1 gene from *Stylosanthes hamata* encoding a root high affinity sulfate transporter enhanced the capacity of *B. juncea* plants to take up chromate [14]. In transgenic tobacco plants, a putative yeast transcriptional activator MSN1 implied in Cr accumulation enhanced tolerance to chromate and higher Cr and S contents compared to the wild-type plants [10].

Cr can influence not only sulfate transport, but also S assimilation. In maize and *B. juncea* plants, the up-regulation of genes coding for enzymes of the S assimilatory pathway was observed in response to Cr, as well as the increase in non-protein thiols (cysteine and glutathione [12,13]. Since Cr seems to be unable to induce phytochelatins [15], the higher production of glutathione might be associated with a role in the reduction of Cr(VI) to the less toxic trivalent form [15,16].

To date, transcriptomic studies focusing on the identification of genes involved in plant response to Cr have not considered the effect of S nutrition on the variation of gene expression profiles [17,18]. Furthermore, the effects of chromate on sulfate transport in selected plant species have been evaluated only in relation to a single sulfate transporter [12,13]. However, the sulfate uptake by plants and the redistribution among organs and between cell compartments occur through several sulfate transporters (SULTR) [19–21]. These transporters are encoded by a large gene family, which can be divided into four closely related groups (SULTR1 to 4), and are characterized by 12 membrane-spanning domains and a STAS (Sulphate Transporter and Anti-Sigma Antagonist) domain at the carboxy-terminus [20,21].

Given the multiple concentration-dependent contrasting effects of Cr on S transport and accumulation, the study of chromate and sulfate interactions attains great interest when plants are being employed for the reclamation of Cr-contaminated substrates. Therefore, the main objectives of this study are: (1) the evaluation of Cr and S accumulation; (2) the analysis of *B. juncea* sulfate transporter gene transcripts with respect to regulation by S/Cr combinations; (3) the detection of other *B. juncea* gene transcripts regulated by S/Cr that may represent possible targets for the genetic engineering of plants to enhance their phytoremediation capacity. Analyses in this investigation were performed on 12-day old *B. juncea* seedlings for a 24-h time course, differently from a previous study performed on younger *B. juncea* seedlings (7-days old) exposed to higher Cr concentration (1 mM) for a longer time period (2 days) [13].

2. Materials and methods

2.1. Plant material

Seeds of *B. juncea* (L.) Czern. (Cv. PI 426314) were surface-sterilized by rinsing in 70% (v/v) ethanol for 30–60 s, then in 5% (v/v) sodium hypochlorite (NaClO) for 30 min while rocking on a platform, and washed in distilled water for 5 × 10 min. The seeds were allowed to germinate and grow for 8 d in half-strength MS agar medium [22] inside a chamber with a 13 h light/11 h dark cycle, air temperature of 20/15 °C, relative humidity of 70/85% and at a photon flux density (PFD) of 280 mol m^{-2} s^{-1}. Germinated seedlings were transferred to 3 L pots (density = 30 plants per pot) and cultivated for 4 d in a thoroughly aerated nutrient solution with the following composition (mM): KH_2PO_4 (40), $Ca(NO_3)_2$ (200), KNO_3 (200), FeNaEDTA (10), B (4.6), Cl (1.1), Mn (0.9), Zn (0.09), Mo (0.01). A portion of the plants was supplemented with 200 μM $MgSO_4$, and part was grown in the absence of S. In the latter case, $MgCl_2$ (200 μM) replaced $MgSO_4$. The nutrient solution in each pot was renewed every two days.

The experimental design for seedling growth was randomized. Seedlings were divided into 4 groups and grown for 24 h under the following conditions: with 200 μM sulfate (+S), without sulfate (−S), with 200 μM sulfate plus 200 μM chromate (+S + Cr), no sulfate plus 200 μM chromate (−S + Cr). Chromium was supplied as potassium chromate (K_2CrO_4). *B. juncea* seedlings were harvested from three pots at each time point (0, 10 min, 1 h, 6 h, 24 h), for a total of 15 pots per each treatment. In order to collect sufficient plant material to perform the analyses, the experiment was replicated five times. After harvest, seedlings were carefully washed with distilled water, dried with blotting paper and immediately frozen with liquid nitrogen and kept at −80 °C for further molecular and physiological analyses.

2.2. Analysis of total Cr and S levels

Foliar and root tissues were dried for 48 h at 80 °C and then digested in nitric acid as described by Zarcinas et al. [23]. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as described by Fassel [24] to determine each digest's elemental concentrations. For each experimental treatment, data obtained were the means of three measurements from fifty plants each and were expressed as mg element kg^{-1} dry weight.

2.3. Analysis of sulfate content

Frozen foliar and root tissues (500 mg) were ground in liquid nitrogen and suspended in 10 mL of distilled water. The samples were incubated for 2 h in a heating block at 85 °C, and the determination of sulfate was performed via HPLC as described by Schiavon et al. [12]. Sulfate content was expressed in mg sulfate kg^{-1} fresh weight and for each treatment data were the means of three measurements with 50 plants in each.

2.4. Determination of thiol content

Thiols were extracted from frozen roots and leaves according to Wirtz and Hell [25] and quantified after derivatization with the thiol-reactive probe monobromobimane (Invitrogen, Germany) as described in Heeg et al. [26]. For each treatment data were the means of three measurements with thirty plants in each.

2.5. Sulfate uptake

Sulfate uptake into roots was carried out using the radioisotope ^{35}S–(SO_4^{2-}) furnished by PerkinElmer Life Sciences (Boston, MA, USA). Sulfate uptake rates were measured in +S and −S plants after the addition of Cr at times 0, 10 min, 30 min, 1 h, 6 h, or 24 h. At the same time points, the sulfate uptake was assayed in Cr-untreated +S and −S plants. Groups of eight plants per each treatment were transferred for 10 min to vessels containing a complete nutrient solution buffered with 15 mM Tris-MES, pH 5.6. Sulfate in the solutions (200 μM $MgSO_4$) was labeled with ^{35}S–(SO_4^{2-}) (5 MBq $mmol^{-1}$). The temperature of the solutions was maintained at 23 °C and a lamp (OSRAM 400 W – HR) with a PFD of 280 μmol m^{-2} s^{-1} provided an adequate light supply. After the uptake period, the measurement of the radioactivity in roots and shoots was performed as described by Quaggiotti et al. [27]. The experiment was replicated three times.