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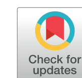
Research article

Impact of increasing chromium (VI) concentrations on growth, phosphorus and chromium uptake of maize plants associated to the mycorrhizal fungus *Rhizophagus irregularis* MUCL 41833



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ABSTRACT

Arbuscular mycorhizal fungi (AMF) associated to plants may represent a promising phyto-remediation avenue due to the widely documented role of these fungi in alleviation of numerous abiotic (e.g. heavy metals) stresses. In the present work, it was the objective to study the dynamics of inorganic phosphorus (Pi) and chromium(VI) (Cr(VI)) and total Cr uptake by the plant-AMF associates *Zea mays* + *R. irregularis* MUCL 41833, under increasing (i.e. 0, 0.1, 1 and 10 mg L^{-1}) concentrations of Cr(VI). The plant-AMF associates were grown in a circulatory semi-hydroponic cultivation system under greenhouse conditions. We demonstrated that Cr(VI) had an hormesis effect on root colonization of maize. Indeed, at 0.1 and 1 mg L^{-1} Cr(VI), root colonization was increased by approximately 55% as compared to the control (i.e. in absence of Cr(VI) in the solution), while no difference was noticed at 10 mg L^{-1} Cr(VI) ($P \leq 0.05$). However, this did not result in an increased uptake of Pi by the AMF-colonized plants in presence of 0.1 mg L^{-1} Cr(VI) as compared to the AMF control in absence of Cr(VI) ($P \leq 0.05$). Conversely, the presence of 1 mg L^{-1} Cr(VI) stimulated the Pi uptake by non-mycorrhizal plants, which absorbed 17% more Pi than their mycorrhizal counterparts ($P \leq 0.05$). In addition, the non-mycorrhizal plants absorbed, in average, 8% more Cr(VI) than the mycorrhizal plants. Overall, our results prompt the hypothesis that in presence of AMF, the regulation of uptake of Cr(VI) and Pi by plant roots is done mostly by the fungus rather than the root cells. This regulated uptake of roots associated to AMF would indicate that the symbiosis could benefit the plants by providing a stable Pi uptake in a Cr(VI) polluted environment.

1. Introduction

Potentially toxic elements (PTEs) such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn), are amongst the most common pollutants, found typically near industrial sites (Baena and Huertos, 2008; Gil-Cardeza et al., 2014; Järup 2003; Meier et al., 2012). At high concentrations in soils, they can lead to changes in the structure and/or functioning of microbial communities (Krishnamoorthy et al., 2015) and cause detrimental effects on ecosystems and human health as they can enter the food chain and pollute drinking water (Taboada, 2018).

Chromium is used in several industrial processes (e.g. leather tanning, alloy and stainless-steel production). Its chemistry in soil is quite complex with two oxidation states, Cr(III) and Cr(VI). Chromium(III) is non-toxic and not readily absorbed by plants. In contrast, Cr(VI) is a Class A carcinogen by inhalation and an acute irritating agent to living cells (Dhala et al., 2013; James, 1996). It is soluble in water in the full pH range, while Cr(III) is prone to adsorb on soil surface or to precipitate as chromium hydroxide in a slightly acidic or alkaline environment (Dhala et al.,2013; James, 1996; Khan, 2001). Hexavalent Cr exist in neutral-to-alkaline soils, principally as a chromate anion (CrO_4^{2-}) or as moderately-to-scarcely soluble chromate salts (e.g. $CaCrO_4$, $BaCrO_4$, $PbCrO_4$) (Dhala et al., 2013; James, 1996).

Chemical reduction of Cr(VI) to Cr(III) is the most common remediation strategy developed until today, most often achieved by using organic waste such as animal manure, Fe(II)-containing salts, or organic acids (Jagupilla et al., 2009; James, 1996; Moon et al., 2009). Though this strategy offers a rapid solution, it could be very expensive for large

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scale treatment and does not guarantee that re-oxidation of Cr(III) to Cr(VI) will not occur (James, 1996; Panda and Sarkar, 2012). In contrast, phytoremediation, using higher plants and/or their associated soil microorganisms is a less expensive, long-lasting, solar-energy dependent and eco-friendly strategy for decontaminating Cr-polluted soils (Ali et al., 2013; Dhala et al., 2013; Ferrol et al., 2016; Vidal et al., 2018). Indeed, it is hypothesized that a number of plants, mostly Cr hyperaccumulators (e.g., *Amaranthus dubius*, *Prosopis laevigata*, *Spartina argentinensis*), are able to convert the highly hazardous Cr(VI) to the relatively less toxic Cr(III) (Ali et al., 2013; Shahid et al., 2017). Moreover, the establishment of vegetation on polluted soils may mitigate erosion and metal leaching. However, data on Cr(VI) alleviation via plants and their associated microorganisms remain few (Gil-Cardeza et al., 2018; Wu et al., 2019) making it necessary to explore their roles in Cr(VI) plant tolerance or uptake/sequestration potential for designing proper Cr(VI) phytoremediation strategies. Among the soil microorganisms that associate to roots, arbuscular mycorrhizal fungi (AMF) are of particular interest. These soil fungi form symbiotic associations with an approximate of 78% of terrestrial plants (Brundett and Tedersoo, 2018). They develop inside the root and grow an extraradical mycelium (ERM) network into the soil. The ERM helps the plants to acquire nutrients (e.g. inorganic phosphorus (Pi), nitrogen (N)). Phosphorus is by far the most studied nutrient absorbed by AM fungal ERM network (Parniske, 2008). It is transported to plants in exchange for carbohydrates and lipids (Keymer et al., 2017; Luginbuehl et al., 2017). In this sense, it has been reported that plants can acquire up to 100% of Pi through AM fungal Pi transporters (Smith and Smith, 2015)). In addition to improving plant nutrition, AMF help plants to resist abiotic stresses such as the excessive concentration of trace elements (TE's) or PTEs (Ferrol et al., 2016) in soils. Therefore their application in remediation of polluted soils may represent an useful approach, possibly combined with other strategies, to increase plant resistance/tolerance to pollutants via different mechanisms (see reviews by Ferrol et al., 2016 and Plouznikoff et al., 2016) and thus improve phytoremediation by immobilization, detoxification and/or transformation of pollutants or by their extraction following increasing translocation from roots to shoots (Vidal et al., 2018; Wu et al., 2019).

The presence of AMF in Cr(VI) polluted soils has been reported (Gil-Cardeza et al., 2014, 2018). An enhanced Cr uptake from pots filled with Cr(III) or Cr(VI) polluted soils was reported for *Prosopis juliflora-velutina* associated with *Glomus deserticola* as compared with non-colonized plants (Arias et al., 2010). Davies et al. (2001) also reported an increased Cr uptake by *Helianthus annuus* roots inoculated with *Rhizophagus intraradices*, in comparison with non-AMF control plants, when grown in pots filled with Cr(III) or Cr(VI) polluted soils. Recently, Gil-Cardeza et al. (2018) reported that an AMF community isolated from *Ricinus communis* rhizosphere in a Cr(VI) polluted soil (named MOR) was more efficient in decreasing Cr(VI) from the soil than an AMF community isolated from *R. communis* rhizosphere in a non-Cr(VI) polluted soil (named PAR). Root colonization was higher in plants grown on the MOR soil than in those grown on the PAR soil. Concomitantly, the root systems of the plants in the MOR soil accumulated more Cr than those in the PAR soil (1840 *vs.* 1540 mg Cr per kg root DW, respectively). However, the mechanisms of Cr(VI) uptake and accumulation by plants and microorganisms, in particular AMF, is not yet completely elucidated. Recently, Gil-Cardeza et al. (2017) observed that the ERM of *R. irregularis* MUCL 41833 associated to *Medicago truncatula* absorbed more HPO_4^{2-} in presence than in absence of CrO_4^{2-}. This suggested that polyphosphates, synthesized by AMF, could be involved in the Cr cellular detoxification mechanisms, as earlier suggested by Wu et al. (2016). However, in this study, conducted under strict *in vitro* culture conditions, only the ERM was exposed to Cr(VI) at a concentration of 2.5 mg L^{-1} of MSR medium (Wu et al., 2016).

The aim of the current work was to evaluate the impact of increasing concentrations (0.1, 1 and 10 mg L^{-1}) of Cr(VI) on growth and Pi and Cr uptake of plants colonized or not by AMF. We chose as a biological model maize plants associated to *R. irregularis* MUCL 41833. The plant-AMF associates were grown in a circulatory semi-hydroponic cultivation system under greenhouse conditions following the method earlier described by Calonne-Salmon et al. (2018) and Garcés-Ruiz et al. (2017). The uptake of Cr(VI) and Pi was evaluated non-destructively in a time course experiment and plant and AMF growth parameters measured at the end of the experiment. In presence of plants associated with the AMF, Pi acquisition was mostly done by AM fungal Pi transporters rather than root epidermal Pi transporters (Ferrol et al., 2016). In addition, root and AM fungal Pi transporters have different Pi affinities (Ferrol et al., 2016). So, if CrO_4^{2-} does indeed enter the cells via HPO_4^{2-} transporters, we can hypothesize that the exposure to different Cr(VI) concentrations will have a differential impact on Pi dynamic in AMF-colonized versus non-colonized plants.

2. Materials and methods

2.1. Biological materials

The AMF *Rhizophagus irregularis* (Błaszk, Wubet, Renker & Buscot) C. Walker & A. Schüßler comb. nov. MUCL 41833 was supplied by the Glomeromycota *in vitro* collection (GINCO - www.mycorrhiza.be/ginco-bel) on the modified Strullu-Romand (MSR) medium (Declerck et al., 1998). The fungus was cultured *in vitro* as detailed in Cranenbrouck et al. (2005). It was subsequently mass-produced during several months on maize (*Zea mays* L. cv. ES Ballade (supplied by the Centre Indépendant de Promotion Fourragère (CIPF – http://www.cipf.be/)) plants in a 13.2 L plastic tray (56.5 cm × 36 cm x 6.5 cm) containing a sterilized (121 °C for 15 min) mixture of vermiculite and sand (w/w, 1/1). Once a week the plants were irrigated with one L of modified Hoagland (Hoagland and Arnon, 1950) solution (i.e. 90% P-impoverished solution – P = 6.245 mg L^{-1}) referred as to Hoagland low-P (see Garcés-Ruiz et al., 2017). The plants were grown under controlled conditions at 22°C/18 °C (day/-night), a relative humidity (RH) of 70%, a photoperiod of 16 h day^{-1} and a photosynthetic photon flux (PPF) of 120 µmol m^{-2} s^{-1}.

Seeds of maize (*Z. Mays* cv. ES Ballade) were supplied by the Centre Indépendant de Promotion Fourragère (CIPF – http://www.cipf.be/). For seed germination, surface-disinfected maize seeds were put on wet paper in the dark at room temperature (~20 °C). The seeds were disinfected with a solution of sodium hypochlorite (8% active chloride, 15 min) and rinsed three times with sterilized (121 °C for 15 min) deionized water (10 min).

2.2. Experimental design

2.2.1. Mycorrhization of the maize plants

Sixty maize seedlings of four days old, germinated as described above, were subsequently planted in two 13.2 L trays to obtain mycorrhizal (M) and non-mycorrhizal (NM) plants. The two plastic trays contained sterilized (121 °C for 15 min) substrate as described in section 2.1. For the M plants, the substrate was inoculated with spores and chopped root pieces sampled from the mass-produced AMF inoculum. For the NM plants, the substrate was inoculated with the same AMF inoculum but sterilized twice (121 °C for 15 min). Fifty mL of sieved (<5 µm aperture) inoculum solution was also inoculated in this tray to reintroduce the microbial community of the growth substrate. Maize seedlings were grown in a growth chamber set at 22/18 °C (day/night), a RH of 70%, a photoperiod of 16 h day^{-1} and a PPF of 120 µmol m^{-2} s^{-1}. The plants were watered each week with one L of Hoagland low-P solution. The intensity of root colonization (%I – see below for method) was estimated after one month, on 3 randomly selected maize plants from both treatments. It was 84 ± 6% for the M treatment, while no colonization was observed in the NM treatment.

2.2.2. Acclimatization period

After 4 weeks of growth in the trays, the maize seedlings were transferred in plastic containers (one plant per container) containing 32 g

of perlite sieved to one mm diam, washed with deionized water and dried. In total, 24 containers for the M treatment and the same number for the NM treatment were prepared. Twelve containers without plants were similarly prepared as a no plant control. At transfer to the containers, the substrate and seed debris were eliminated from the roots of the plants with deionized water. At that time, the height of the maize plants in the M and NM treatments did not differ significantly according to the equivalence test ($P \leq 0.05$) and reached 66.2 ± 2.4 cm and 66.5 ± 2.6 cm for the M and NM treatments, respectively.

The sixty containers were randomly disposed in the greenhouse. Aluminum foil was fold around each container and it surface was enclosed with black-coated raw quartz granulates (Dekoline, Belgium) to prevent algae development. The plants were then acclimatized for 2 weeks at 25°C/18 °C (day/night), a RH of 38%, a photoperiod of 16 h day^{-1} and a PPF of 120 μmol m^{-2} s^{-1}. Each container was irrigated with 200 mL of Hoagland low-P solution every 2 days.

2.2.3. Chromium (VI) concentrations

Hoagland low-P solutions supplemented with Cr(VI) (used as $K_2Cr_2O_7$) at concentrations 0.1, 1 and 10 mg L^{-1} were prepared. The concentrations were chosen considering the maximum accepted accumulation in water and soil (i.e. 0.1 mg L^{-1} for irrigation water and 8 mg kg^{-1} in dry soil) following the regulation in Argentina (Regulative order 389/93, law 24051).

2.2.4. Experimental set-up

The experimental set up consisted in a circulatory semi-hydroponic (S–H) cultivation system as recently described by Garcés-Ruiz et al. (2017). Prior to the start of the experiment, all containers were irrigated with Hoagland low-P solution to set the same nutrient concentration in all of them. The duration of the experiment was 3 weeks with one series of sampling done at week 1 and 3 only. The circulatory system was initiated at the start of each week, at a speed of 7.4 mL min^{-1} and maintained for 48 h, with fresh Hoagland low-P solution, with or without the addition of the 3 Cr(VI) concentrations. In order to asses the initial Cr (total Cr and Cr(VI)) and initial Pi concentration in the nutrient solution, 15 mL from the one L solution was sampled from each bottle prior the beginning of the circulatory system (time 0 – T0). Three other samplings of 15 mL were done at 9, 21 and 39 h (i.e. T9, T21 and T39, respectively).

Six replicates were considered for each treatment: NM plants grown in absence (NM^{NoCr}) or in presence of increasing (0.1, 1 and 10 mg L^{-1}) concentrations ($NM^{0.1Cr}$, NM^{1Cr}, NM^{10Cr}) of Cr(VI) and M plants grown in absence (M^{NoCr}) or in presence of increasing (0.1, 1 and 10 mg L^{-1}) concentrations ($M^{0.1Cr}$, M^{1Cr}, M^{10Cr}) of Cr(VI). Three non-vegetated containers without Cr or with increasing concentrations of Cr(VI) (0.1, 1 and 10 mg L^{-1}) were used as no plant controls.

2.3. Dynamics of Cr and Pi uptake by AMF-colonized and non-colonized maize plants grown under increasing concentrations of Cr(VI)

Chromium and Pi uptake dynamics were analyzed indirectly by determining the total Cr, Cr(VI) and Pi concentrations in the Hoagland low-P solution at T0, T9, T21 and T39 as explained above. The collected nutrient solutions were stored at 4 °C in the dark before Cr and Pi analysis.

In order to avoid possible oxidation/reduction reactions in the samples, Cr(VI) concentration was measured within the first 48 h of the sampling by diphenylcarbazide (DPC) photometric method. Briefly, the DPC technique allows the quantification of Cr(VI) in the medium since the solution changes to pink in presence if Cr(VI) and DPC. The intensity of color is positively correlated to the concentration of Cr(VI) (James et al., 1995). Thus, for Cr(VI) quantification, a calibration curve was done (i.e. 0.4, 0.8, 1, 2, 4, 6, 8 μg Cr(VI)). In addition, total Cr and Pi were quantified by inductively coupled plasma atomic emission spectrometer (ICP-AES). Six mL of deionized water was added to four mL of the Hoagland low-P nutrient solution and then acidified with 20 μL HNO_3 at 65% (Merck, Germany) before ICP-AES analysis. Data obtained (in ppm) were converted in mg L^{-1}.

Pi depletion values were standardized according to those obtained by their respective no plant control containers and Pi concentration at time T0, following the formula described in Garcés-Ruiz et al. (2017):

$$[P]_X = [P]_X \text{ quantified with ICP-AES at time T} + ([P]_{blank} \text{ at } T_0 \text{ - } [P]_{blank} \text{ at T})$$

where:

[P] = Pi concentration in the solution
X = sample
blank = non-vegetated containers respective to the Cr(VI) concentration considered
T = time considered (9, 21, or 39 h after the start of the circulatory system)
T0 = time zero (just prior the beginning of the circulatory system)

Net Pi uptake was determined from the depletion of Pi in a Hoagland low-P solution circulating through the plant containers.

2.4. Plants growth parameters, AMF root colonization and total Pi and Cr concentrations and contents in plants

At the end of the experiment, the plants were harvested and shoot and root fresh weights (SFW and RFW, respectively) were measured. For each plant, an approximate of 200 mg of root fresh tissues was sampled randomly, stored in liquid nitrogen and then transferred to -80 °C for determination of acid and alkaline phosphatase activities. The shoot and roots dry weights (SDW and RDW, respectively), were further estimated after drying in an oven at 50 °C for 120 h. After drying, each root system was separated in two identical parts to evaluate AMF root colonization (see below) and total P and Cr concentrations in plant tissues.

2.4.1. Acid and alkaline phosphatase activity in roots

One hundred mg of fresh root tissues was grinded with liquid nitrogen in a mortar and 1 mL of sucrose triton extraction buffer (STEB) at pH 8 was added (Sen and Hepper, 1986). The homogenate was then transferred to a 2 mL Eppendorf tube and centrifuged at 16,000 rpm at 4 °C for 20 min. The supernatant was transferred to a new 2 mL Eppendorf and stored at -80 °C until enzymatic activities determination. The acid and alkaline phosphatases enzymatic activities were quantified according to the protocol described by Labidi et al. (2011). Total protein was determined with Lowry method (Lowry et al., 1951) following the manufacturer instructions (Total protein Kit, Micro Lowry, Peterson's modification, Sigma-Aldrich).

2.4.2. Total Pi and Cr in plant tissues

Two hundred mg of shoot or roots was sampled from the dried material, ground separately in a grinder and incinerated at 500 °C for 3 h. The minerals were extracted with 2mL of HNO_3 followed by incubation in 1 mL $HClO_4$. Once the $HClO_4$ had evaporated completely, the minerals were re-suspended in 2 mL of HCl:HNO_3 (3:1 v/v) and diluted with ultrapure water (Millipore, France) to a final volume of 25 mL. The solution was filtered with filter paper N°1 (pore diameter = 11 μm, Whatman, UK) in a 25 mL volumetric flask, before analysis. Total Pi and Cr concentrations were converted from ppm to mg kg^{-1} and shoots and roots content of Pi and Cr were determined according to the dry weight of shoots and roots.

2.4.3. AMF root colonization

Dry roots were placed in Falcon tubes (Sarstedt, Germany) and rehydrated for 48 h in deionized water. The re-hydrated roots were put in 25 mL of KOH 10% and incubated at 70 °C in a water bath for 30 min. The KOH was removed, roots were vigorously washed with tap water followed by a final wash with HCl 1%. Maize roots were

stained with ink 2% (Parker blue ink, USA). The staining procedure consisted in an incubation for one h at 70 °C in a water bath with 25 mL of an ink 2% solution containing HCl 1%. The roots were finally rinsed and stored in deionized water before observation (Walker, 2005).

For AMF root colonization quantification, the frequency of root colonization (%F), the intensity of root colonization (%I) and arbuscule abundance in mycorrhizal root system (%a) were calculated. Twenty root fragments of ~10 mm length were mounted on microscope slides and examined under a compound microscope (Olympus BH2, Olympus Optical, GmbH, Germany) at 20–40 x magnifications. In addition. The %I was calculated as follows: $(v + 5w + 30x + 70y + 95z)/(v + w + x + y + z)$, where v, w, x, y, z are the number of root fragments containing a proportion (i.e. v: <1%, w: 1–10%, x: 11–50%, y: 51–90%,z: > 90%) of AMF structures (i.e. hyphae, arbuscules or vesicles/spores; adapted from Plenchette and Morel, 1996). The %a was calculated with the formula: (100A3+50A2+10A1)/mb where A3, A2 and A1 are the number of root fragments containing a proportion (i.e. A3:>50%, A2:10–50%, A1<10%) of arbuscules, and mb is the total number of the 20 root fragments containing AMF structures. The %F was calculated as the percentage of root fragments that contained AMF structures.

2.5. Statistical analysis

The statistical analysis was conducted using INFOSTAT (Di Rienzo et al., 2011) free edition. Chromium(VI), total Pi and Cr concentrations in Hoagland low-P nutrient solution were analyzed with t-test for repeated measures ($P \leq 0.05$). Shoot and root dry weights (SDW and RDW), RDW/SDW ratios, total Pi and Cr concentrations in plant tissue and content together with the enzymatic activities were analyzed by a two-way ANOVA ($p \leq 0.05$). Differences between the averages of the data were analyzed with the Bonferroni post-test (all determinations were analyzed at $P \leq 0.05$, except for alkaline phosphatase activity which was analyzed at $P \leq 0.1$). The %I, %F and %A were analyzed with a one-way ANOVA ($P \leq 0.05$). Multiple comparisons were made by a multiple range Tukey test ($P \leq 0.05$). Data that did not assume the assumptions for homoscedasticity and normality were transformed as follows: shoot and root Cr content with log10; RDW and acid phosphatase activity with natural logarithm; %I and %F were transformed with arcsine $\sqrt{}$. Assumptions for homoscedasticity and normality were met for the rest of the data analyzed.

3. Results

3.1. Impact of Cr(VI) on growth and phosphatase activities of maize plants associated or not to *Rhizophagus irregularis*

Chromium(VI) significantly impacted SDW, RDW, root/shoot ratio and phosphatases (i.e. ALP and ACP) activities, irrespective of the mycorrhizal status of the plants (Table 1). At the end of the experiment (i.e. after 3 successive periods of exposure to Cr(VI) in the circulating Hoagland low-P solution), the SDW and RDW of the plants in the M and NM treatments receiving the highest concentration of Cr(VI) (i.e. 10 mg L^{-1}) was significantly lower as compared to the plants in the control and the two other Cr(VI) treatments. Conversely, no significant difference in SDW and RDW was observed between the plants in the control and Cr(VI) treatments at concentrations 0.1 and 1 mg L^{-1}, with the exception of the SDW of the NM plants in the control treatment that was significantly higher to the SDW of the M plants in presence of 1 mg L^{-1} Cr(VI). Whatever the mycorrhizal status, the root/shoot ratio of the plants in the treatments receiving Cr(VI) was significantly higher as compared to the root/shoot ratio of those in the control treatment. Similarly, the root/shoot ratio of the NM and M plants grown in presence of 0.1 and 1 mg L^{-1} of Cr(VI) was significantly lower as compared to those receiving 10 mg L^{-1} of Cr(VI). No significant difference was noticed in root/shoot ratio between the plants in the treatments receiving 0.1 and 1 mg L^{-1} of Cr(VI), irrespective of their mycorrhizal status.

Root colonization by the AM fungus did not impact the root/shoot ratio of the plants, while it significantly impacted SDW and RDW (Table 1). Even if SDW and RDW were generally non-significantly lower for the plants in the M treatments as compared to their respective NM control treatments, it was significantly different at 10 mg L^{-1} of Cr(VI), as proven by the Bonferroni post-hoc test. No significant interactions between the factors ‘AM fungus treatment’ and ‘Cr treatment’ were observed for SDW, RDW and root/shoot ratio (Table 1).

Chromium(VI) significantly impacted ALP and ACP activities in roots of maize (Table 1). Indeed, at the end of the experiment, the ALP activity was significantly lower in the roots of the M and NM plants in the treatment receiving 10 mg L^{-1} of Cr(VI) as compared to the roots of the M and NM plants in the control treatment or in the treatment receiving 0.1 and 1 mg L^{-1} of Cr(VI). Similarly, the ALP activity in the M roots of plants receiving 1 mg L^{-1} of Cr(VI) was significantly lower than that of M control roots. The ALP activity in the roots of the M plants in the

Table 1. Shoot and root dry weights (SDW, RDW, respectively), root/shoot ratio, root alkaline and acid phosphatase (ALP and ACP respectively) activities (U mg prot^{-1}) of maize plants associated (M) or not (NM) to *Rhizophagus irregularis* MUCL 41833 in containers connected to a circulatory semi-hydroponic cultivation system receiving Hoagland low-P solution without Cr(VI) or supplemented with increasing (0.1, 1 and 10 mg L^{-1}) concentrations of Cr(VI). Measurements were done after 30 days of growth in the containers.

Cr(VI) (mg L^{-1})	Mycorrhizal Treatment	SDW (g)	RDW (g)	Root/shoot	ALP (U mg prot^{-1})	ACP (U mg prot^{-1})
0	NM	8.2 ± 0.4^{a}	1.8 ± 0.1^{a}	0.23 ± 0.01^{c}	3.4 ± 0.1^{a}	18 ± 2^{a}
	M	7.6 ± 0.7^{ab}	1.8 ± 0.2^{a}	0.24 ± 0.01^{c}	3.2 ± 0.1^{ab}	18 ± 1^{a}
0.1	NM	6.6 ± 0.4^{ab}	1.9 ± 0.1^{a}	0.30 ± 0.02^{b}	3.1 ± 0.2^{ab}	17 ± 1^{a}
	M	6.4 ± 0.4^{ab}	1.8 ± 0.1^{a}	0.29 ± 0.02^{b}	2.6 ± 0.2^{bc}	15 ± 2^{ab}
1	NM	6.8 ± 0.6^{ab}	1.9 ± 0.2^{a}	0.28 ± 0.01^{b}	2.9 ± 0.1^{ab}	12 ± 1^{bc}
	M	5.8 ± 0.3^{b}	1.7 ± 0.1^{a}	0.29 ± 0.01^{b}	2.2 ± 0.2^{c}	11 ± 1^{c}
10	NM	2.1 ± 0.2^{c}	0.9 ± 0.05^{b}	0.42 ± 0.03^{a}	0.9 ± 0.1^{d}	24 ± 5^{a}
	M	1.4 ± 0.2^{c}	0.6 ± 0.07^{c}	0.44 ± 0.04^{a}	0.8 ± 0.2^{d}	17 ± 3^{ab}
p value						
AM fungus treatment		**0.0364**	**0.0045**	0.5105	**0.0020**	0.1436
Cr treatment		**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0009**
AM fungus treatment * Cr treatment		0.7842	0.0677	0.9073	0.1396	0.5840

Data are expressed as means ± SE (N = 6). Values with the same lower-case letters in a column do not differ significantly at $P \leq 0.05$ (two-way ANOVA, Bonferroni post-test). ALP values differ significantly at $P \leq 0.1$. Significant P values are highlighted in bold.

treatment receiving 1 mg L^{-1} of Cr(VI) was significantly lower as compared to the activity measured in the roots of the NM plants in the treatment receiving the same concentration of Cr. Surprisingly, the ACP activity was significantly lower in roots of the plants in the treatment receiving 1 mg L^{-1} of Cr(VI) as compared to the others treatments, in both NM and M plants, whereas it remained similar between M and NM plants in the treatments receiving 0.1 and 10 mg L^{-1} of Cr(VI) as compared to the respective controls.

Root colonization by the AM fungus significantly reduced ALP activity. This activity was generally higher in the roots of the NM plants as compared to the M ones, irrespective of the concentration of Cr(VI). This was particularly marked for the plants in the treatment receiving 1 mg L^{-1} of Cr(VI), according to the Bonferroni post-hoc test. At this concentration, the ALP activity measured in the M plants was significantly lower to the one in the NM plants. Conversely, the ACP activity was not affected by the factor 'AM fungus treatment'. No interactions between the factors 'AM fungus treatment' and 'Cr treatment' were observed on the ALP and ACP activities.

3.2. Impact of Cr(VI) on root colonization by *Rhizophagus irregularis*

Root colonization (i.e. %I, %F and %a), measured at the end of the experiment, differed between the treatments (Figure 1). The %I was significantly higher in the plants of the treatments receiving 0.1 and 1 mg L^{-1} of Cr(VI) as compared to control plants and those receiving 10 mg L^{-1}. The %F was significantly lower in the plants of the control treatment as compared to those in the treatments receiving Cr(VI), that did not differ among them. The %a was significantly higher in the plants of the treatments receiving 0.1 and 1 mg L^{-1} of Cr(VI) as compared to the plants in the control treatment and those receiving 10 mg L^{-1} of Cr(VI). No significant difference was observed between the plants in the control treatment and those receiving 10 mg L^{-1} of Cr(VI).

3.3. Effects of *Rhizophagus irregularis* on Cr(VI) and total Cr uptake by maize plant

Short-term dynamics of Cr(VI) and total Cr uptake by maize plants from the Hoagland low-P solution were determined at four time points of circulation (0, 9, 21 and 39 h) at week 1 (14 days after transfer of the plants into the containers - Figure 2 A–C) and 3 (28 days after transfer of the plants into the containers - Figure 2 D–F). Whatever the week of measurement, an uptake of Cr(VI) and total Cr was generally observed for the M and NM plants of the treatments receiving 0.1 and 1 mg L^{-1} of Cr(VI). After 39 h of circulation (week 1), the NM and M plants in the treatment receiving 0.1 mg L^{-1} of Cr(VI) took up ~35% of Cr(VI) and ~20% of total Cr from the Hoagland low-P solution, while at week 3, ~60% of Cr(VI) and total Cr were taken up from the solution. In the case of total Cr a higher Cr uptake was observed at 9 h, as compared to Cr uptake at 21 and 39 h (Figure 2A). After 39 h of circulation (week 1), the NM and M plants in the treatments receiving 1 mg L^{-1} of Cr(VI) took up ~15% of Cr(VI) and total Cr from the Hoagland low-P solution, while at week 3–20% of Cr(VI) and total Cr were taken up from the solution. For the M and NM plants in the treatment receiving 10 mg L^{-1} of Cr(VI), Cr(VI), total Cr concentrations remained generally unchanged in the Hoagland low-P solution at time 9, 21 or 39 h in comparison with 0 h, irrespective of the time of observation (i.e. week 1 or 3). Indeed, neither NM nor M plants of the treatment receiving 10 mg L^{-1} of Cr(VI) took up more than 8% of Cr(VI) and total Cr.

The M plants in the treatments receiving 0.1 and 10 mg L^{-1} of Cr(VI) took up similar quantity of Cr(VI) and total Cr from the circulating Hoagland low-P solution as compared with their NM controls, irrespective of the time of measurement (i.e. 9, 21 or 39 h). Conversely, an increased Cr uptake was measured for NM maize plants in the treatment receiving 1 mg L^{-1} of Cr(VI) as compared to the M plants. During the 1st week of circulation of the nutrient solution containing 1 mg L^{-1} of Cr(VI), the difference was statistically significant only for total Cr at 39 h, whereas the difference was statistically significant for both Cr(VI) and total Cr during the 3rd week of circulation at 39 h.

3.4. Impact of Cr(VI) on Cr concentration and content in maize plants associated or not to *Rhizophagus irregularis*

The addition of Cr(VI) to the Hoagland low-P circulating solution impacted Cr accumulation in shoot as well as in roots, whereas the association to *R. irregularis* and the interactions between the AMF and Cr(VI) treatments did not (Table 2). Indeed, at the end of the experiment,

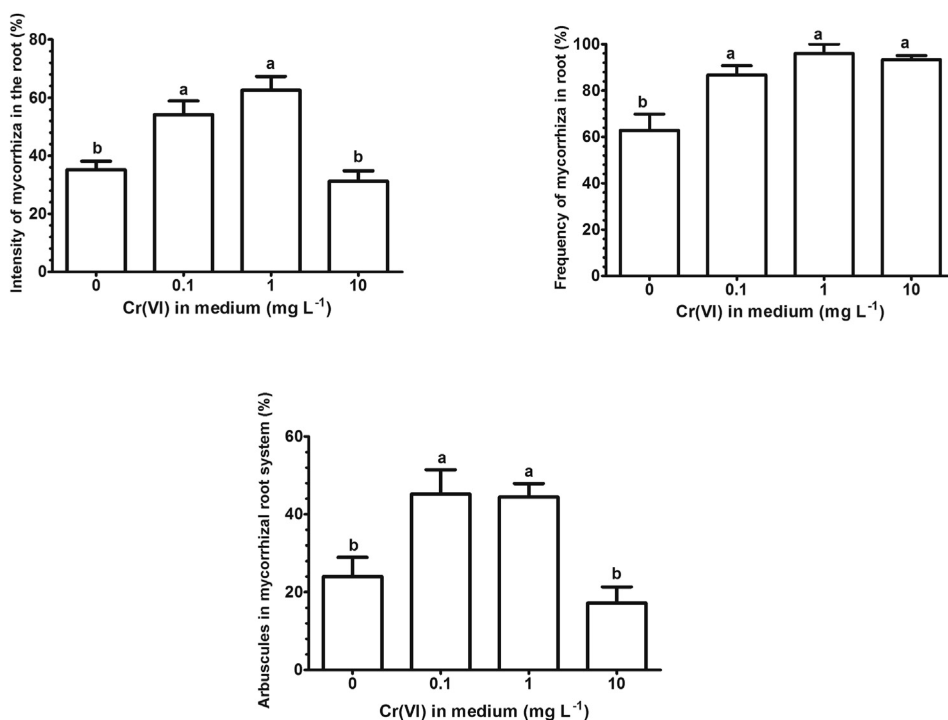

Figure 1. Root colonization intensity (%I), frequency (%F) and arbuscules abundance (%a) in maize plants associated to *Rhizophagus irregularis* MUCL 41833 in containers connected to a circulatory semi-hydroponic cultivation system receiving Hoagland low-P solution without Cr(VI) or supplemented with increasing (0.1, 1 and 10 mg L^{-1}) concentrations of Cr(VI). Measurements were done after 30 days of growth in the containers. Data are expressed as means ± SE (N = 6). Values with the same lower-case letters in a graph do not differ significantly at $P \leq 0.05$ (one-way ANOVA; multiple comparisons were made by a multiple range Tukey test).

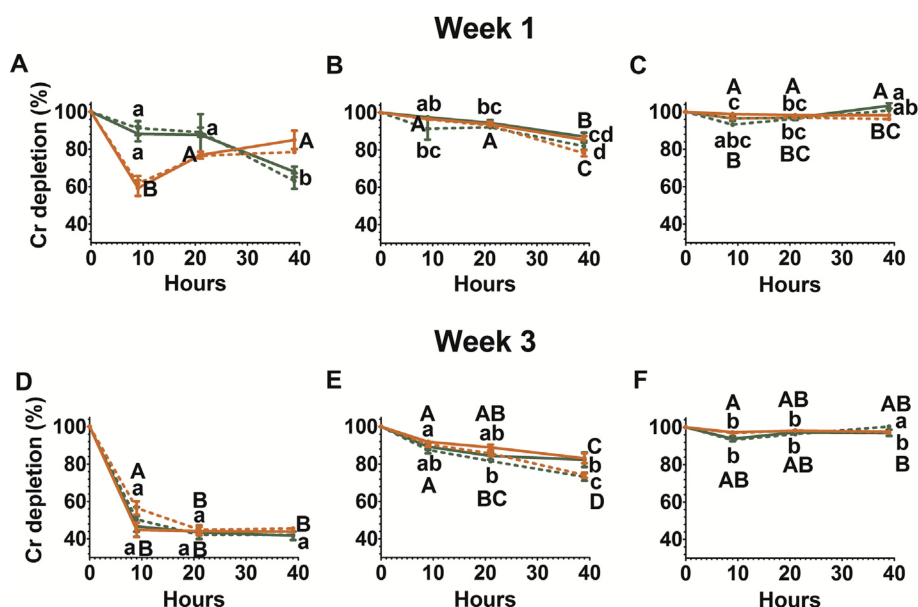

Figure 2. Short-term Cr(VI) (green lines) and total Cr (orange lines) depletion analysis (expressed as the % of the initial Cr(VI) concentration in the nutrient solution) in Hoagland low-P solution circulating in containers with maize plants colonized (full lines) or not (dashed lines) with *Rhizophagus irregularis* MUCL 41833. Plants were exposed to increasing (0.1 (A and D), 1 (B and E) and 10 (C and F) mg L^{-1}) concentrations of Cr(VI) in the Hoagland low-P solution supplied to the plants for 48 h once a week during three successive weeks. Cr(VI) and total Cr in the Hoagland low-P were determined during week 1 of observation (A, B and C) and week 3 of observation (D, E and F). Data are expressed as means ± SE (N = 5). The presence of different letters indicates a significant difference between treatments (Cr(VI): lower-case letters, total Cr: capital letters), as determined by a t-test for repeated measures ($P \leq 0.05$).

Table 2. Total Pi and Cr shoot and root concentrations (μg g^{-1} of DW) and contents (μg $plant^{-1}$) of maize plants associated (M) or not (NM) to *Rhizophagus irregularis* MUCL 41833 in containers connected to a circulatory semi-hydroponic cultivation system receiving Hoagland low-P solution without Cr(VI) or supplemented with increasing (0.1, 1 and 10 mg L^{-1}) concentrations of Cr(VI). Measurements were done after 30 days of growth in the containers.

Cr(VI) (mg L^{-1})	Mycorrhizal Treatment	Cr in shoot		Cr in root		Pi in shoot		Pi in root	
		μg g^{-1}d.w.	μg * shoot	μg g^{-1}d.w.	μg * root	μg g^{-1}d.w.	μg * shoot	μg g^{-1}d.w.	μg * root
0	NM	0.5 ± 0.2^{bc}	4.2 ± 1.5^{a}	0.6 ± 0.2^{d}	1.0 ± 0.3^{d}	2255 ± 94^{ab}	18.4 ± 0.6^{a}	1008 ± 73^{c}	1.9 ± 0.2^{bc}
	M	0.4 ± 0.2^{c}	3.1 ± 1.3^{a}	0.8 ± 0.2^{d}	1.4 ± 0.3^{d}	2411 ± 52^{a}	18.3 ± 1.7^{a}	1722 ± 119^{ab}	3.1 ± 0.3^{a}
0.1	NM	0.6 ± 0.2^{bc}	3.6 ± 1.3^{a}	12.1 ± 1.3^{c}	23.3 ± 2.5^{c}	2158 ± 92^{abc}	14.2 ± 1.1^{b}	982 ± 78^{c}	1.9 ± 0.3^{bc}
	M	0.6 ± 0.2^{bc}	4.0 ± 1.5^{a}	10.3 ± 1.8^{c}	19.2 ± 3.7^{c}	2062 ± 92^{bc}	13.1 ± 0.9^{b}	1518 ± 129^{b}	2.8 ± 0.4^{ab}
1	NM	0.8 ± 0.2^{bc}	5.4 ± 1.5^{a}	122 ± 18^{b}	240 ± 43^{b}	1867 ± 43^{c}	12.8 ± 1.2^{b}	1682 ± 140^{ab}	3.2 ± 0.2^{a}
	M	1.0 ± 0.2^{ab}	16.2 ± 7.6^{a}	133 ± 14^{b}	220 ± 23^{b}	2048 ± 40^{bc}	11.8 ± 0.7^{b}	2024 ± 54^{a}	3.4 ± 0.3^{a}
10	NM	4.2 ± 0.6^{a}	9.1 ± 1.6^{a}	793 ± 113^{a}	648 ± 65^{a}	1938 ± 57^{bc}	4.1 ± 0.3^{c}	1364 ± 44^{bc}	1.2 ± 0.1^{c}
	M	4.0 ± 0.6^{a}	5.5 ± 0.9^{a}	819 ± 149^{a}	491 ± 123^{ab}	2046 ± 81^{bc}	2.8 ± 0.3^{c}	1573 ± 77^{b}	0.9 ± 0.2^{c}
AM fungus treatment		0.5540	0.8114	0.5448	0.5374	0.0954	0.1890	**< 0.0001**	**0.0029**
Cr treatment		**< 0.0001**	**0.0073**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
AM fungus treatment *Cr treatment		0.2776	0.2473	0.4197	0.2008	0.2233	0.9341	0.0583	**0.0144**

Data are expressed as means ± SE (N = 6). Values with the same lower-case letters in a column do not differ significantly at $P \leq 0.05$ (two-way ANOVA, Bonferroni post-test). Significant P values are highlighted in bold.

the Cr concentrations in the shoots of M and NM maize plants in the treatments receiving 10 mg L^{-1} of Cr(VI) significantly increased as compared with their respective controls. When reported to the DW, the Cr content in shoot of both NM and M plants in the treatments receiving 1 and 10 mg L^{-1} of Cr(VI) slightly increased, but non-significantly, in comparison with their respective controls. In roots, the Cr concentration and content of both NM and M plants markedly increased with increasing concentration of Cr(VI) added to the Hoagland low-P solution. This effect was significant in the roots of plants in the treatments receiving 0.1 mg L^{-1} of Cr(VI) as compared with the roots of plants in the control treatment. Roots of M plants in the treatments receiving 1 and 10 mg L^{-1} of Cr(VI) contained similar Cr contents but significantly higher Cr contents than plants in the treatment receiving 0.1 mg L^{-1} of Cr(VI) or in the control treatment.

3.5. Impact of Cr(VI) on Pi uptake by maize plants associated or not to *Rhizophagus irregularis*

Short-term dynamics of Pi uptake by maize plants from the Hoagland low-P solution were determined at four time points of circulation (0, 9, 21 and 39 h) at week 1 (14 days after transfer of the plants into the containers - Figure 3 A–D) and 3 (28 days after transfer of the plants into the containers - Figure 3 E–H). Whatever the week of measurement, the uptake of Pi from the Hoagland-low P solution significantly increased over time, with the exception of NM and M plants of the treatments receiving 10 mg L^{-1} of Cr(VI) at week 3. This uptake was more pronounced during week 3 as compared to week 1. Indeed, during week 1, both M and NM plants grown in the control treatment or in the treatments receiving increasing concentrations of Cr(VI) took around 40% of the initial Pi from the nutrient solution, whereas at week 3, between 0% (plants receiving 10 mg L^{-1} of Cr(VI)) and 95% (plants in the control treatment) of the initial Pi was taken up, irrespective of the mycorrhizal status of the plants. During week 1, the Pi uptake remained similar between the NM and M plants in the control treatment as well as those receiving 0.1 mg L^{-1}. Conversely, significant differences were observed for the plants in the treatments receiving 1 and 10 mg L^{-1} of Cr(VI). Indeed, the NM plants in the treatment receiving 1 mg L^{-1} of Cr(VI) took up a significant higher Pi quantity from the nutrient solution than the M ones at 21 and 39 h and at all observation times for those receiving 10 mg L^{-1}. During week 3, the Pi uptake remained similar between the plants in

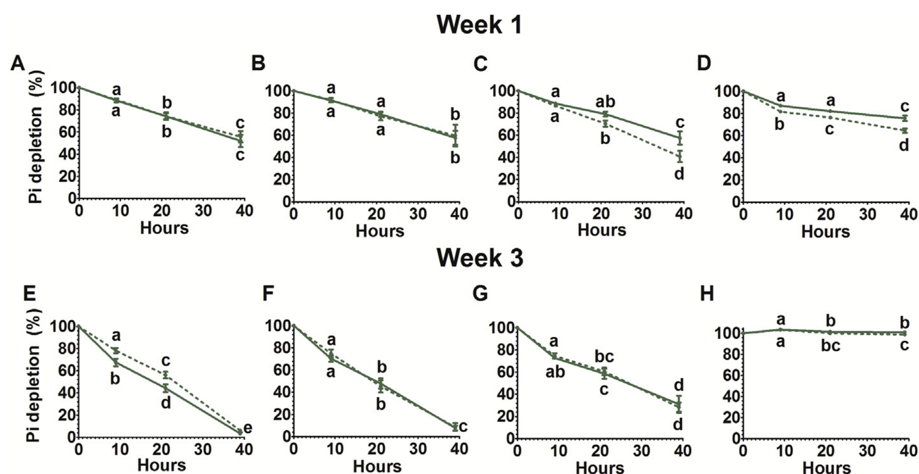

Figure 3. Short-term inorganic phosphorus (Pi) depletion (expressed as the % of the initial Pi concentration in the nutrient solution) in Hoagland low-P solution circulating in containers with maize plants colonized (full lines) or not (dashed lines) with *Rhizophagus irregularis* MUCL 41833. Plants were exposed to increasing (0 (A and E), 0.1 (B and F), 1 (C and G) and 10 (D and H) mg Cr(VI) L^{-1}) concentrations of Cr(VI) in the Hoagland low-P solution supplied to the plants for 48 h once a week during three successive weeks. Pi in the Hoagland low-P was determined during the week 1 of observation (A, B, C and D) and the week 3 of observation (E, F, G and H). Data are expressed as means ± SE (N = 5). The presence of different letters indicates a significant difference between treatments as determined by a t-test for repeated measures ($P \leq 0.05$).

the NM and M treatments receiving Cr(VI), irrespective of the concentration. Conversely, the M plants in the control treatment took up 15 and 20% more Pi from the circulating Hoagland low-P solution as compared to their respective NM controls at 9 and 21 h respectively. In addition, the quantity of Pi taken up in the Hoagland low-P by both NM and M plants in the treatments receiving 0.1 mg L^{-1} of Cr(VI) at 21 h was similar to the quantity of Pi taken up by the M plants at 21 h, in the treatment without Cr(VI) (45 *vs.* 44% of Pi, respectively).

3.6. Impact of Cr(VI) on total Pi concentration and content in maize plants associated or not to Rhizophagus irregularis

The addition of Cr(VI) to the Hoagland low-P solution impacted the Pi concentration and content in shoot of maize plants, while the association to *R. irregularis* as well as the interaction between the AMF and Cr(VI) treatments did not (Table 2). The Pi concentration was significantly lower in the shoots of the NM and M plants of the treatments receiving 1 and 10 mg L^{-1} of Cr(VI), as compared to the NM and M plants in the control treatment. Similarly, the total Pi content in shoots was significantly higher in the plants of the control treatment as compared to those receiving Cr(VI) whatever the concentration and irrespective of the mycorrhizal status. No difference was observed in Pi content of the plants in the treatment receiving 0.1 and 1 mg L^{-1} of Cr(VI).

The Pi concentration in the roots of the M plants of the control treatment was similar to the M plants in the treatments receiving Cr(VI), irrespective of the concentration. Conversely, the Pi concentration in the roots of the NM plants in the treatment receiving 1 and 10 mg L^{-1} of Cr(VI) was significantly higher as compared to the NM plants in the control treatment. The association to *R. irregularis* also strongly affected the root Pi concentration and content. In the control treatment or in the treatment receiving 0.1 mg L^{-1} of Cr(VI), the concentration of Pi was significantly higher in the roots of the M plants as compared to their respective NM controls. The interaction between the AMF treatment and Cr treatment had no effect on the Pi concentration in roots. Conversely, a significant interaction was observed between both factors for the Pi content in roots, indicating that the amount of Pi in roots depended both on the association to *R. irregularis* and to the Cr(VI) concentration in a non-linear way. For instance, the presence of *R. irregularis* had a positive impact on Pi content in control while it did not have an impact on Pi content when maize plants were grown in presence of Cr(VI).

4. Discussion

Remediation of chromium-contaminated sites is generally based on chemical processes. However in the last decade, phytoremediation, a technique combining plants with their associated microorganisms, has emerged as a realistic, environmental-friendly and cost-effective approach to address the problem of Cr(VI) pollution. As such, the application of AMF may represent a promising avenue because of the many benefits to plants reported to date (Plouznikoff et al., 2016). In the present study, maize plants associated to the AMF *R. irregularis* MUCL 41833 were grown in a circulatory semi-hydroponic cultivation system in absence or under increasing (0.1, 1 and 10 mg L^{-1}) concentrations of Cr(VI) applied three consecutive weeks for a duration of 39 h each time. Maize was chosen because of its enhanced Pi uptake in presence of *R. irregularis* MUCL 41833 in a similar semi-hydroponic model system (Garcés-Ruiz et al., 2017) and because of its tolerance to Cr(VI) reported by several authors (Guo et al., 2013; Maiti et al., 2012; Shanker et al., 2005; Soni et al., 2013).

Whatever the presence/absence of AMF, SDW and RDW of maize plants exposed to the highest Cr(VI) concentration (i.e. 10 mg L^{-1} of Cr(VI)) were significantly lower as compared to the plants grown in absence of Cr(VI), suggesting that this Cr(VI) concentration had a strong detrimental impact on plant development. In addition, at the highest Cr(VI) concentration, plants associated to the AMF had lower SDW and RDW, although only significant for RDW, as compared to their NM controls. This suggested that the highest concentration of Cr(VI) had a strong detrimental impact on the plant metabolism (i.e. nutrients uptake and probably carbon metabolism and reallocation) that could not be compensated by an increasing nutrient uptake often-reported with plants associated to AMF. It could be hypothesized that at the highest concentration of Cr(VI), the C (i.e. the photosynthates) balance between plant parts and fungus was not counterbalanced by a sufficient provision of nutrients from fungus to plant, resulting in a slower growth as compared to the NM plants.

Noticeably, the reverse was observed for the root/shoot ratio, with the highest values recorded at 10 mg L^{-1} of Cr(VI). Root/shoot ratio is considered to be a health index that reflects biomass allocation between above- and below-ground parts of plants and thus differential investment of photosynthates and minerals between those organs. How environmental stresses impact root/shoot ratio remains difficult to predict because plants adjust their growth in function of environmental conditions that may vary from biome to biome. In a meta-data analysis, these authors reported that plant tend to allocate more biomass to roots under more stressful, low-nutrient conditions (Qi et al., 2019). One possible explanation to the increased root/shoot ratio of maize plants exposed to Cr(VI) could be a negative effect on xylem loading reflected as a deficient mineral translocation from root to shoot. Xylem loading is a fine regulated mechanism, so it is quite difficult to predict the effect of a stress (Taiz and Zeiger, 2010). However, in our study, Pi content in shoot was negatively affected by the increasing concentrations of Cr(VI) while Pi content in root was not. These observations demonstrates a negative effect of Cr(VI), even at low concentrations, on Pi translocation to shoot. An increased root/shoot ratio was also reported in maize plants under

drought stress (Benjamin et al., 2014) and in *Arabidopsis thaliana* under Pi deficiencies (Hermans et al., 2006). On the contrary, Wu et al. (2014) reported a decreased of root/shoot ratio in *Taraxacum platypecidum* and *Cynodon dactylon* when grown in presence of Cr(VI) in soil (notice that in our experiment Cr(VI) was in Hoagland low-P solution while in the former was stabilized in soil). The plant tissue dry weights and the root/shoot ratio of the maize plants exposed to 0.1 and 1 mg L^{-1} of Cr(VI) did not differ suggesting that these two Cr(VI) concentrations had a similar effect on plant growth.

Chromium(VI) exposure affected AMF root colonization. The frequency (%F) of colonization increased with the concentration of Cr(VI). This was also reported by Wu et al. (2014) for Dandelion at 5 and 10 mg Cr(IV) per kg of soil. One possible explanation is that the stress produced in presence of Cr(VI) inhibited root growth more drastically than fungal growth, at least at the two higher Cr(VI) concentrations, leading to a relative greater root colonization in Cr(VI) exposed plants as compared to the control plants (qualitative morphological root changes were observed at 1 and 10 mg L^{-1} of Cr(VI), lateral roots were less numerous than in their respective NM controls, data not shown). Roots exposed to 10 mg L^{-1} of Cr(VI) and control roots (not exposed to Cr(VI)) had the same %I and %A. The %I and %A were the highest in roots exposed to 0.1 and 1 mg L^{-1} of Cr(VI). The percentage of arbuscules in the root system was the double than in the control plants, suggesting that the symbiosis was more active in these roots. These findings suggests an hormesis effect since root colonization was stimulated at the lowest concentrations of Cr(VI) (i.e. 0.1 and 1 mg L^{-1}) of Cr(VI) and inhibited at the highest (i.e. 10 mg L^{-1}). Even if a higher percentage of arbuscules was quantified, this was not reflected in a significant higher accumulation of Pi neither in roots nor in shoots as compared to the control. In addition, the increase of %I and %A in roots exposed to 0.1 and 1 mg L^{-1} of Cr(VI) suggested that the AMF used in this study (*R. irregularis* MUCL 41833) was tolerant to low or moderate Cr(VI) concentrations. This AMF has often been reported in PTE polluted areas, i.e. in presence of Cr(VI) (Gil-Cardeza et al., 2018), As, Cd and Zn (Krishnamoorthy et al., 2015), As (Schneider et al., 2013), Pb and Zn (Zarei et al., 2010). Moreover, Gil-Cardeza et al. (2017) demonstrated that this specific strain, MUCL 41833, was tolerant to Cr(VI) during a short-time exposure (24 h).

Total Cr and Cr(VI) depletion from the Hoagland low-P nutrition solution differed at the three Cr(VI) concentrations. At 10 mg L^{-1} of Cr(VI), the NM and M plants did not take up more than 8% of the total Cr and no differences were observed between week 1 and week 3. At the lowest Cr(VI) concentration (0.1 mg L^{-1}), the NM and M plants took up more Cr from the solution at week 3, as compared to week 1. Because plant growth seemed not significantly impacted at this concentration (plant height was similar between the controls and NM and M plants - data not shown), it is probable that the higher Cr depletion in the Hoagland low-P solution was due to a greater root system at week 3, as compared to week 1 (NM and M shoots were ~30% higher). At the first exposure to 0.1 mg L^{-1} Cr(VI) (week 1) total Cr uptake by the plants was higher at the first hours (i.e. 9 h). Afterwards, an increase in the total Cr concentration of the nutrient solution was determined until 39 h of circulation. A possible explanation to this could be that an adsorption of Cr on roots could have taken place at the beginning of the exposure followed by leakage to the nutrient solution. This behavior was only observed when total Cr was determined with ICP-AE; it was not detectable when Cr(VI) was measured with the DFC colorimetric method. When exposed to 1 mg L^{-1} of Cr(VI), NM plants depleted significantly more Cr than M plants. At the end of the experiment (week 3, 39 h), NM maize plants depleted 27% while M maize plants depleted 18%. As explained by Gil-Cardeza et al. (2017), no specific CrO_4^{2-} transporters have been reported in plant roots. It has been hypothesized that CrO_4^{2-} enters root cells via HPO_4^{2-} or SO_4^{2-} transporters (Fargasova, 2012; Kleiman and Cogliatti, 1997; Oliviera et al., 2016). Though mycorrhizal formation induced the expression of HPO_4^{2-} (*Gint*PT) and SO_4^{2-} (*LjSultr1;2*) transporters (Fiorilli et al., 2013; Giovannetti et al., 2014), the influence of CrO_4^{2-} exposure on the expression of these transporters has not been reported yet. Thus, the higher CrO_4^{2-} depletion observed in NM plants could be due to a stimulation of root HPO_4^{2-} and/or SO_4^{2-} transporters by CrO_4^{2-}. Despite the different uptake, Cr concentration in plant tissue remained similar between NM and M plant roots and shoot. The lack of differences was probably due to the short time of Cr(VI) exposure (3 times, for 48 h, and three weeks between the first exposure and the harvest). Shoot Cr(VI) concentration and content were the same between all Cr(VI) concentrations, including no exposed to Cr(VI) controls, confirming that maize is not a Cr hyperaccumulator.

The Pi uptake of plants was strongly impacted by Cr(VI) concentration and presence or absence of AMF. In absence of Cr(VI), Pi depletion in the nutrient solution followed a linear decrease both at week 1 and 3 for the M and NM plants. This depletion was higher at week 3 and differed between the M and NM plants (the M plants depleted more Pi at 9 and 21 h), probably because at week 3 the plants occupied a larger volume of the substrate, even larger in presence of AMF. This corroborated the results of Calonne-Salmon et al. (2018) and Garcés-Ruiz et al. (2017). In presence of Cr(VI) this uptake was impacted markedly both in presence and absence of AMF. When maize plants were exposed to 0.1 mg L^{-1} of Cr(VI), Pi depletion was similar between NM and M plants. In addition, Pi depletion values of roots in NM plants exposed to 0.1 mg L^{-1} of Cr(VI) were similar to Pi depletion values of roots in the M control plants strongly suggesting that the presence of Cr(VI) indeed stimulated Pi uptake. In presence of 1 and 10 mg L^{-1} of Cr(VI) the NM maize plants depleted significantly more Pi than the M plants at week 1, while at week 3, no differences were noticed. These findings support the hypothesis that root HPO_4^{2-} transporters were induced by CrO_4^{2-} while HPO_4^{2-} transporters induced by mycorrhizal formation (*Gint*PT) were not. One mg L^{-1} of Cr(VI)-exposed plants depleted 25% less Pi than no Cr(VI) plants, indicating a toxic effect at this Cr(VI) concentration. In accordance, at 10 mg L^{-1} of Cr(VI) exposure, no Pi depletion was observed. In link with the impact of Cr(VI) on the Pi uptake, root ALP and ACP enzymatic activities were affected by the pollutant. ALP activity decreased with the increase of Cr(VI) concentration while ACP activity had a bi-phasic behavior (hormesis effect): the activity decreased only when plants were exposed to the intermediate concentration of 1 mg L^{-1} of Cr(VI). On the other hand, the lack of influence of the AMF on maize roots ALP and ACP activities in the present work has already been reported (Dodd et al., 1987).

Concomitantly to Pi uptake, Cr(VI) exposure impacted Pi accumulation in shoot. Indeed, Pi accumulation in shoot of plants exposed to 10 mg Cr(VI) was 85% lower than in absence of Cr(VI). Interestingly, the M control plants and those exposed to 0.1 mg L^{-1} of Cr(VI) accumulated significantly more Pi in roots than NM plants, while no significant differences were noticed at 1 and 10 mg L^{-1} of Cr(VI). Moreover, the Pi concentration and content in NM roots were higher at these Cr(VI) concentrations as compared to plants without Cr(VI) and at 0.1 mg L^{-1} Cr(VI), also supporting the hypothesis that the presence of Cr(VI) can modulate Pi uptake in NM maize plants. An enhanced Pi uptake could alleviate Cr toxicity. Indeed, Qian et al. (2013) reported that the algae *Chlorella vulgaris* absorbed more Pi to alleviate the toxicity of Cr.

5. Conclusion

We demonstrated that the exposure to non-lethal polluting concentrations of Cr(VI) (i.e. 0.1 and 1 mg L^{-1}) stimulated the mycorrhizal association between maize and *R. irregularis* MUCL 41833 and that the stimulation neither increased Pi uptake nor decreased Cr(VI) uptake. Conversely, the presence of 1 mg L^{-1} Cr(VI) stimulated the Pi uptake by NM roots and NM roots depleted more Cr(VI) than M roots. Overall, our results prompt the hypothesis that in presence of AMF and Cr(VI), the regulation of the uptake of Cr(VI) and Pi by plants roots is done by AMF rather than root cells. This meticulously regulated uptake of roots in symbiosis with AMF would indicate that the symbiosis could benefit the plants by providing a stable Pi uptake in a Cr(VI) polluted environment. It

will be interesting to further investigate the HPO_4^{2-} and *Gint*PT transporters activation and expression in roots of NM and M plants in presence or absence of Cr(VI).

Declarations

Author contribution statement

Gil-Cardeza María Lourdes, Calonne-Salmon Maryline: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Declerck Stéphane: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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