



Cerium negatively impacts the nutritional status in rapeseed



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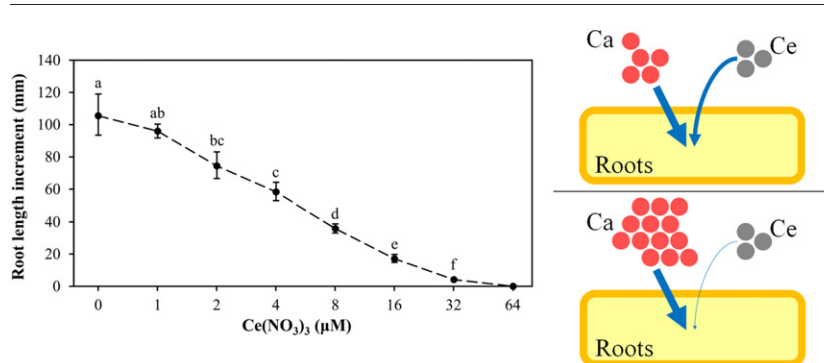
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HIGHLIGHTS

- Ce fertilization is controversial and Ce movement pathways to roots and shoots are unknown.
- Ce, Ca (Lithosphere) and rapeseed (Biosphere) interaction was assessed in hydroponics.
- Ce was without beneficial effects and already toxic at very low concentrations (2 μM).
- Ce accumulation was inhibited in a non-competitive way by high Ca concentrations in the nutrient solution.
- The use of Ce-containing fertilizers in agriculture should be avoided.

GRAPHICAL ABSTRACT



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ABSTRACT

Cerium (Ce) has been reported to be both beneficial and harmful to plants. This contradiction deserves explanation in the light of increased anthropogenic release of Ce in the environment.

Ce tolerance and accumulation were evaluated in hydroponically cultivated *Brassica napus* L. (rapeseed). Ce and other nutrient concentrations were measured with increasing Ce concentration in the nutrient solution. Moreover, Ce and calcium (Ca) accumulation were evaluated at different Ca and Ce concentrations in nutrient solution and a Michaelis-Menten type inhibition model considering Ce and Ca competition was tested. Plants were also sprayed with Ce solution in Ca-deficient media.

Ce decreased the growth and root function, which affected shoot nutritional status. Calcium was the most severely inhibited nutrient in both roots and shoots. High Ca concentrations in the nutrient solution inhibited Ce accumulation in a non-competitive way. Moreover, phosphorus (P) precipitated Ce inside root cells. Ce spraying did not alleviate Ca deficiency symptoms and the results were critically compared to the available literature.

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1. Introduction

Despite being classified as a rare earth, Ce is the 25th most abundant element and its presence in the soil ranges approximately from 2 to 150 $\mu\text{g g}^{-1}$, with mean values of 50–66 $\mu\text{g g}^{-1}$ (Greenwood and Earnshaw, 1984; El-Ramady, 2010; Emsley, 2011). Cerium and other rare earths

(REs) have the potential to accumulate progressively in soil due to their increased use in a variety of modern industries and in the last 30 years their wide use as fertilizers in Chinese agriculture (Hu et al., 2004; Tyler, 2004; El-Ramady, 2010; Emsley, 2011; Ramos et al., 2016). Due to elevated concentrations of REs in phosphate fertilizers, Ce can accumulate in agricultural soils outside China too (Kanazawa and Kamitani, 2006; Ramos et al., 2016). For example, it has been estimated that in Brazilian soils 12,000 t of Ce were added through phosphate fertilizers in 2014 (Ramos et al., 2016). However, the increase of REs in soil is usually small and large amounts are easily washed away

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from upper soil horizons through rain of watering (Tyler, 2004). Nevertheless, REs supplied as fertilizers or those from other anthropogenic sources are more soluble and reactive than REs from the soil pool. Rare earths pollution can therefore be considered an upcoming environmental problem (Tyler, 2004).

In a natural environment Ce bioavailability will be enhanced by low pH, and it is likely that it will be complexed with dissolved organic carbon (Tyler and Olsson, 2002). Instead, Ce bioavailability will decrease in soils with high phosphate, which can precipitate Ce as non-soluble CePO_4 (Diatloff et al., 1993; Diatloff et al., 1996). An approximation of Ce solubility in soil would be its concentration in soil solution, which had a range of 0.01–0.51 μM in unamended Australian soils (Diatloff et al., 1996) and of 0.005–0.19 μM in Swedish non-fertilized forest (Tyler and Olsson, 2002). Soluble Ce concentrations in fertilized or contaminated soils could be much higher.

Most plants in unfertilized soils have been reported to avoid REs accumulation, with a plant to soil concentration ratio far lower than unity, and with roots usually having higher concentrations than shoots (Tyler, 2004). However, it has been reported that *Dryopteris erythrosora* contain up to 30 $\mu\text{g g}^{-1}$ (0.2 $\mu\text{mol g}^{-1}$) of Ce in leaves, which is a factor 10 to 100 higher than in non-accumulator ferns from the same place (Ozaki et al., 2000). Another fern, *Dicranopteris dichotoma* (synonymous with *D. linearis*) has the highest concentrations of total REs (and Ce) in leaves ever reported for a vascular plant thus far (Ichihashi et al., 1992; Wang et al., 1997). Ichihashi et al. (1992) reported that *D. dichotoma* can contain up to 140 $\mu\text{g g}^{-1}$ (1 $\mu\text{mol g}^{-1}$) of Ce in leaves. This is confirmed by data derived from Wang et al. (1997), which also showed a high Ce root to shoot translocation ratio (>80), with a mean value of c. 508 $\mu\text{g g}^{-1}$ (3.6 $\mu\text{mol g}^{-1}$) Ce in leaves, which was on average seven times higher than the concentration in the soil. Robinson et al. (1958) also observed a high Ce concentration (on average 172 $\mu\text{g g}^{-1}$ i.e. 1.2 $\mu\text{mol g}^{-1}$) in leaves of hickory trees (*Carya* sp.). However, in the above-cited papers it is not possible to exclude contamination of leaves by dust containing REs deposited by wind and experimental confirmations are still lacking.

In nutrient solutions with a realistic range of Ce concentrations (0.37–1.31 μM) maize (*Zea mays*) fresh and dry weight and nutritional status were much less affected than those of mungbean (*Vigna radiata*) at $\geq 0.2 \mu\text{M}$ (Diatloff et al., 1995a, 1995b; Diatloff et al., 2008). In maize low Ce concentrations appeared to have beneficial effects on root elongation and dry weight (Diatloff et al., 1995b). However, no beneficial effects were observed for shoot and total dry biomass, and Ce inhibited root elongation at concentrations higher than 1.31 μM (Diatloff et al., 1995a). Hu et al. (2002a, 2002b) observed no beneficial effects of Ce on growth of common wheat (*Triticum aestivum*) at 3.6 μM in nutrient solution, while only harmful effects such as decreased root growth and nutrient unbalance were noted at concentrations $\geq 7.1 \mu\text{M}$. Similarly, Wang et al. (2007) observed that Ce induced oxidative stress in *Hydrilla verticillata* already at 10 μM Ce in the nutrient solution. Liu et al. (2012) who cultivated rice (*Oryza sativa*) in agar, and Shyam and Aery (2012) who cultivated cowpea (*Vigna unguiculata*) in Ce-spiked soil, observed a beneficial effect of Ce on plant growth, i.e., at low concentrations (till 100 μM in rice and c. 18 μM in cowpea) Ce improved growth, but plants were negatively affected at higher concentrations. Similar beneficial effects have been reported for fresh and dry weight in *Ginkgo biloba* cell suspensions (Chen et al., 2015). The reasons for these beneficial effects are unknown. Fashui and co-authors demonstrated an increased photosynthetic rate, in particular the photochemical activity of photosystem II, its oxygen evolving rate, and improved growth and chlorophyll content in spinach after submersing seeds in Ce solution before germination, and then spraying the leaves with Ce at c. 15 μM (Fashui et al., 2002) and 81 μM (Fashui et al., 2005). Similar beneficial effects on photosynthesis have also been obtained with Ce concentrations at 5, 10 and 30 μM (Xiaoqing et al., 2007). The authors attributed these effects to a possible replacement of Mg by Ce atoms in their coenzyme sites. Indeed, Chen et al. (2000), working in vitro

with Mg-less bathing solutions, found that at low concentrations ($\leq 6 \mu\text{M}$) Ce promoted ribulose 1,5-bisphosphate carboxylase (RuBPCase) activity by replacing Mg coenzyme activity. Instead, higher Ce concentrations ($\geq 8 \mu\text{M}$) inhibited RuBPCase activity in vitro. Bakou et al. (1992) and Bakou and Ghanotakis (1993) observed inhibition of the oxygen evolution rate in photosystem II (PSII) in vitro, owing to replacement of Ca with REs in plants treated with very high REs concentrations (2 mM and 800 mM). Negative or no effects after spraying Ce at high concentrations have also been observed (Diatloff et al., 1999; Guo et al., 2007). Diatloff et al. (1999), who used commercial fertilizer containing Ce and La, and in a parallel experiment a spray with Ce and La of analytical grade in a ratio of 1 to 0.7, found no beneficial effects on the growth and yield of mungbean and maize after applying a range of high concentrations (c. 0.2–8.8 mM Ce). However, necrotic patches on maize and small necrotic spots on mungbean were observed at the highest application rates (c. 4.4 and 8.8 mM Ce). Guo et al. (2007) reported a significantly decreased content of Ca, K and Mg and a time dependent increase of Ce in horseradish (*Armoracia rusticana*) roots after applying 1000 $\mu\text{g Ce l}^{-1}$ (c. 7 mM) on the leaf surface, suggesting that high Ce concentrations can disturb the homeostasis of these major cation nutrients.

Although Ce is typically 3+ charged and Ca 2+, both elements have similar atomic radii (Ca = 197 pm, Ce = 181.8 pm) and their cations have similar Shannon-Prewitt effective ionic radii (for example for coordination number 6 Ca^{2+} has an ionic radius of 100 pm whereas Ce^{3+} of 101 pm). It can thus be supposed that Ce might partly replace or interact with Ca and its binding sites. Indeed Chao et al. (2008, 2009) and Huang et al. (2008) showed that spraying with 15 μM Ce improved plant growth, photosynthesis, RuBPCase activity, nitrogen metabolism, and the antioxidative response, and alleviated calcium-deficiency symptoms in spinach, grown in Ca-deficient media. However, a mechanistic insight into the effects of Ce and its interaction with Ca metabolism is still lacking.

The objectives of the present research are therefore, (i) to assess the toxicity or any beneficial effects of Ce on rapeseed (a widely cultivated crop for its oil production) in controlled experimental conditions under increasing and realistic Ce concentrations in nutrient solution; (ii) to evaluate the rapeseed response to Ce exposure in terms of Ce accumulation and translocation and its effects on the plant mineral status; (iii) to investigate the nature of Ca/Ce interference regarding their uptake and root-to-shoot translocation.

2. Material and methods

2.1. Plant culture and experimental conditions

Seeds of *Brassica napus* L. var. 'Pulsar' (rapeseed) from University of Udine (Italy) were sown in a garden peat soil (Typical Typ 2 Gebr. Brill Substrate GmbH and Co. Georgsdorf, Germany) and left for ten days in a growth chamber. Seedlings were then transferred to aerated hydroponic culture, in 1-L polyethylene pots (one plant per pot) containing a modified half-strength Hoagland's solution composed of 3 mM KNO_3 , 2 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.5 mM MgSO_4 , 20 μM $\text{Fe}(\text{Na})\text{-EDTA}$, 1 μM KCl , 25 μM H_3BO_3 , 2 μM MnSO_4 , 2 μM ZnSO_4 , 0.1 μM CuSO_4 and 0.1 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ in demineralised water buffered with 2 mM MES, pH 5.5, adjusted with KOH. Nutrient solutions were renewed after five days and plants were grown in a growth chamber (22: 18 °C, day: night; light intensity 220 $\mu\text{E m}^{-2} \text{s}^{-1}$, 12 h day⁻¹; relative humidity 75%).

2.2. Ce tolerance testing

After ten days of pre-culture, plants were transferred to the test solution, which was of the same background composition as the pre-culture solution, but without $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Fe}(\text{Na})\text{-EDTA}$, to avoid precipitation of CePO_4 and Ce-EDTA complex formation, owing to

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