

THE EFFECT OF DIFFERENT VERTICAL ZINC DISTRIBUTION ON THE EARLY ROOT DEVELOPMENT AND ZINC ACCUMULATION OF *BRASSICA NAPUS*

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Abstract

The early development of the root system is highly important for the plants' whole life; it is regulated by a complex system which integrates endogenous and environmental signals. Heavy metals (HMs) as exogenous factors have a different, concentration-dependent effect on the developing root: low concentrations cause stress-induced morphogenic response (SIMR, adaptation mechanism, more branched, shallower roots), while higher concentrations inhibit growth. Plants encounter heavy metals in many different ways, not only the widely studied homogenous distribution. Thus investigation of the effect of vertical distribution of HMs on the root development of crops has a great importance. In our experiments, to be able to determine and characterise the exact effect of excess zinc (Zn) on the root growth of *Brassica napus* L., several different layouts were used in soil-filled rhizotrons. This setup allowed us to examine the root growth of rapeseed in a practically 2D system, submitted to heterogeneously layered Zn-contaminated soil.

Introduction

The early development of the root system is crucial in terms of the life of a plant; besides ensuring physical stability for the whole plant, it is responsible for water and nutrient uptake and also might be practically (phytoremediation) relevant. It has a noteworthy plasticity: due to different stress conditions its architecture can change to favour seedling vigour and yield stability.

The delicate balance of the endogenous signal system responsible for the development of the root can be affected by various environmental stimuli, such as the excess of essential or non-essential HMs. Heavy metal contamination of soils and water is an existing and growing problem. This has partly originated from agricultural processes, such as excessive use of fertilisers or application of sewage [1].

HMs at low concentrations are able to induce the morphological and physiological adaptation of the root system called stress-induced morphogenic response. SIMR is a special combination of inhibition of primary root growth and induction of lateral root development, resulting in a shallower but horizontally more extensive root architecture, which most likely provides an enhanced stress tolerance [2-3], however this conjecture needs further investigations. On the other hand, HMs at high concentration lead to growth inhibition due to their phytotoxic effect by altering the most important plant physiological and metabolic processes [4]. The effect of HM excess on plants depends on numerous factors, including their mobility and their capability of bioaccumulation. Zn is a redox inactive essential microelement, with low mobility and accumulation capability in plants [5]. Zn, in low quantities as a micronutrient is essential for the normal development of plants, however in excess it might be toxic for plants and other eukaryotic organisms as well. Zn stress causes growth alterations and cell wall modifications, together with loss of functionality of enzymes and decreased photosynthesis.

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Experimental

Custom-made plexi panels were ordered and assembled into 15 cm wide, 30 cm tall and 1,6 cm thick rhizotrons, using polifoam sheets and screws with wing nuts. The front panel is made of 3 mm thick, anti-glare, 100% transparent plastic, while the back panel is a 3 mm thick non-transparent black sheet; the thickness of the soil layer inside the rhizotron is 1 cm.

The rhizotrons are filled with Klasmann Potgrond P blocking substrate (100% frozen through black peat with a fine structure of maximum 8 mm size, pH 6,0; 210 mg N/l; 240 mg P₂O₅/l) mixed with 20% sand; the initial water content is set to 70%. According to preliminary experiments 10 and 500 ppm Zn supplementation as treatment were chosen as SIMR-inducing and growth-inhibiting concentrations, respectively. Several heterogeneous setups were assembled (top/bottom half or top/middle/bottom third, respectively): control/10 ppm Zn (layout 1); 10 ppm Zn/control (layout 2); control/10 ppm Zn/control (layout 3); control/500 ppm Zn (layout 4); 500 ppm Zn/control (layout 5); control/500 ppm Zn/control (layout 6); 500 ppm Zn/10 ppm Zn/control (layout 7). *Brassica napus* L. (rapeseed) seeds were pre-germinated for 24 hours on 26°C and then transferred to the soil surface of the pre-filled rhizotrons. In the first 48 hours after the seeding, the young seedlings were covered with transparent plastic foil to provide optimal humidity, then the growing plants were supplemented with 10 ml distilled water in every two days. Seedlings were grown for 10 days, then the rhizotrons were scanned, disassembled and the roots were cleaned for further examination.

For visualisation of Zn, root tips were equilibrated in PBS buffer (137 mM NaCl, 2,68 mM KCl, 8,1 mM Na₂HPO₄, 1,47 mM KH₂PO₄, pH 7,4) then incubated with 25 µM Zinquin (ethyl (2-methyl-8-p-toluenesulphonamido-6-quinolyloxy)acetate) in PBS, for one hour at room temperature, in darkness, as described by Sarret et al. [6]. Roots dyed with Zinquin were investigated under a Zeiss Axiovert 200M inverted microscope (Carl Zeiss, Jena, Germany) equipped with a high resolution digital camera (Axiocam HR, HQ CCD, Carl Zeiss, Jena, Germany) and filter set 49 (exc.: 365 nm, em.: 445/50 nm). Fluorescence intensities (pixel intensity) in the meristematic zones of the root tips were measured on digital images using Axiovision Rel. 4.8 software within circles of 50 µm radii.

The results are expressed as mean ± SE by using Microsoft Excel 2016 and Student's t-test were used (*P≤0.05, **P≤0.01, ***P≤0.001). All experiments were carried out at least two times. In each treatment at least 10 samples were measured.

Results and discussion

Roots grown in layout 1 and 2 showed approximately the same growth and Zn-contents, the low Zn-treatment did not alter the morphology significantly. In layout 3 root morphology did not differ between the zones; however, Zn-content of the root tips were higher in the 10 ppm Zn supplemented zone. The difference between the Zn-accumulation capacity of the plants grown in the two- and three-layered systems can be explained by that in the layout 3 the growing root met with the Zn supplement in a younger developmental state; it is known that the HM accumulation capacity of the young, metabolically more active plant organs are high.

Upon encountering the growth-inhibiting Zn treatment the rapeseed roots showed different behaviour in the different layouts. When the germinating seedling faced the control soil at first (layout 4), it was able to develop a longer root system, and it also penetrated the 500 ppm Zn supplemented zone; however, there the growth stopped soon. In the opposite scenario, upon confronting the high Zn treatment zone first inhibited root growth in a much higher degree, the root could only slightly enter the lower control zone. Zn content of the root tips changed according to the soil Zn content, though there was a difference between the Zn-accumulation of the two layouts: in layout 4 the difference between the Zn accumulation of the control and 500 ppm zones were bigger than in layout 5. A possible explanation behind this difference can be that in layout 4 the growing root met with the control soil at its younger age and remained more active metabolically, while in layout 5 the growing root was in a stressed state and tried to exclude Zn.

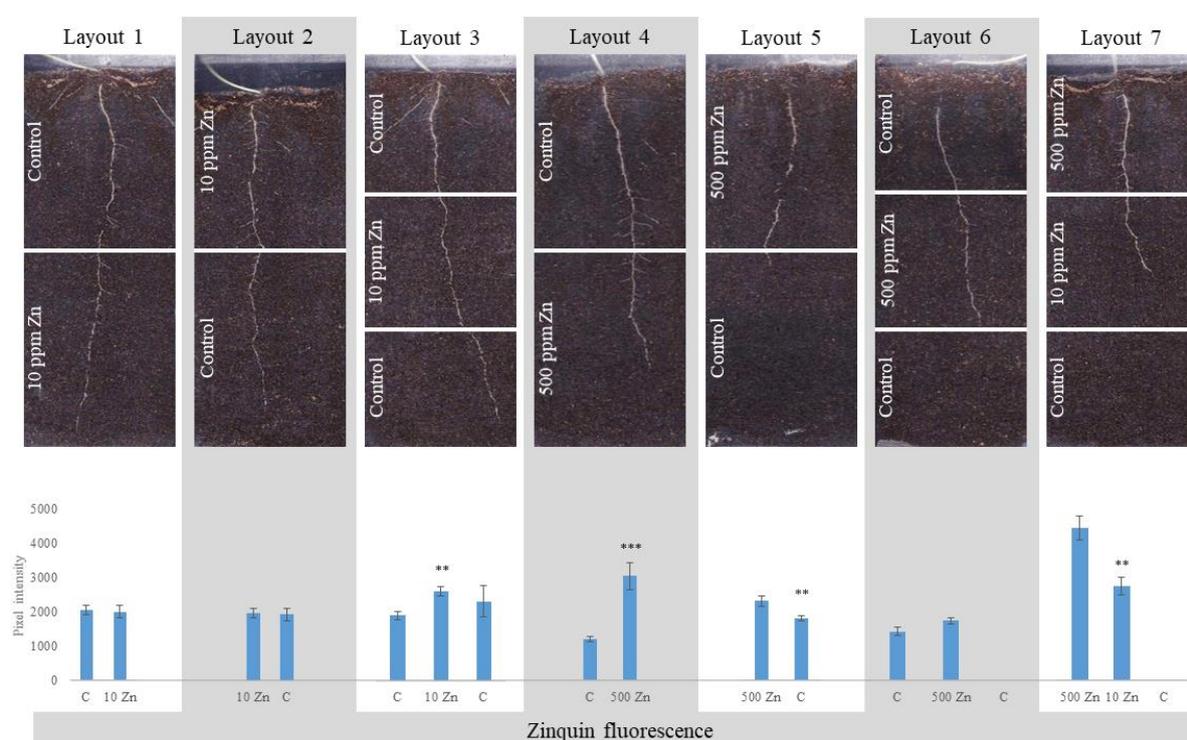


Figure 1. Representative images of the root growth in the different heterogeneous rhizotron system layouts and the Zn content-dependent Zinquin-fluorescence values in the root tips of the corresponding soil zone. Significant differences according to Student's t-test ($n = 10$, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$) between each layouts' control and Zn-containing zones are indicated.

Layout 6 can be considered as the combination of the previous two, and the developing roots showed mixed growth characteristic: since the emerging root met the control soil at first it was able to penetrate and grow in the high Zn containing zone, though it did not enter the lower control layer. While in layout 5 the “young” root was able to grow through the high-Zn-zone, in layout 6 the growing root was relatively older upon encountering the same layer, resulting in the arrest of root growth and exclusion of Zn from its root tips.

In layout 7 we wanted to simulate a real-life Zn-contaminated soil, where the upper layer contained high amount of Zn, decreasing until control level in the lower zones. Similar to layout 5, the root system was able to grow through the 500 ppm Zn layer and entered the 10

ppm Zn zone, but could not reach the control at the bottom of the rhizotron. The roots accumulated far the highest amount of Zn in the entire experiment, and while the Zn content of the root tips grown in the 10 ppm layer were lower, it was still relatively high compared to the control levels. Seedlings in their early developmental stage receive plenty of nutrients from the seed, the root has a high metabolic activity and growth rate, which enabled the developing root to grow through the 500 ppm Zn zone, but the high Zn concentration ultimately caused the inhibition of root development in the subsequent, low-Zn-containing soil layer.

Conclusion

Results obtained from the heterogeneous Zn distribution experiments point towards the presumption that the growth inhibiting effect of the 500 ppm Zn treatment depends on the developmental stage of the rapeseed seedlings. When the root encountered the high Zn dose in a younger stage, it did not suffer growth inhibition, while the toxic effect of Zn is more pronounced on older roots. Considering the Zn accumulation, there was no close connection between the applied treatment and the Zn content of the root tips in all applied layouts.

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