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Research article

Structural and functional alterations induced by two sulfonamide antibiotics on barley plants

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ABSTRACT

Synthetic veterinary medicines are introduced routinely in the environment after animal treatment to prevent and control infectious diseases and up to 80% the administered dose can be excreted unaltered. As a consequence, the soil is the environment most contaminated by such molecules. However, information about their implications on the growth of vegetal organisms is still scarce. With the aim of better elucidating the effects of veterinary antibiotics on plants, barley was grown in a nutrient solution containing 40 μM (about 11,500 μg L^{-1}) of two well-known sulfonamide antibiotics, sulfadimethoxine (SDM) and sulfamethazine (SZ). After 15 d of treatment, the effects on root apparatus were particularly evident, while the photosynthetic tissues remained almost unaffected. SDM and SZ stimulated root hairs and lateral root development a few mm behind the root tips. In particular, from a structural point of view, treated plants showed root shortening and an advanced differentiation in comparison to controls, later confirmed using light microscopy. At a functional level, the two active molecules were found to induce root electrolyte release, such as K^+, possibly due to an impairment of membrane permeability. The research concludes that sulfonamides can have profound effects on morphology and functionality of roots of crop plants. As these alterations might have consequences on their productivity, further studies are necessary to assess effects on plants at laboratory and field conditions.

1. Introduction

Veterinary antibiotics are used in the livestock industry mainly for the therapeutic treatment of sick animals, as well as for illness prevention and to increase feed efficiency [1]. Such medicines constitute 70% of consumed veterinary medications [2] and several studies have shown that 30–80% of an antibiotic dose can rapidly pass through the gastrointestinal tract of animals in an unaltered state [3–5]. Consequently, antibiotics present in animal manure can enter the agricultural soil while animals are grazing, or through manure application [6]. In addition, the use of some antibiotics as pesticides (e.g., oxytetracycline and sulfonamides) in crop production could contribute to the presence of residues in soil [7,8]. Furthermore, the consumption of plant-derived food in humans and livestock nutrition could lead to antibiotic exposure [9]. In fact, organics with a log K_{ow} between 0.5 and 3 are hydrophobic enough to move through the lipid bilayer of membranes and are still water soluble enough to travel into the cellular fluids [10].

Unfortunately, environmental contamination by these molecules has raised concerns only recently. Mean concentrations reported in literature of sulfonamide antibiotics found in soil are around 200 μg kg^{-1} [8,11], but can reach up to 200 mg L^{-1} in manure [12]. However, concentrations reported in literature may be underestimated due to the high mobility of veterinary medicines in the environment [8].

Since the soil is the most exposed environmental compartment to such kind of pollutants [13], the potential impact on plant growth and development needs to be assessed. Uptake of pharmaceuticals into plants has been previously reported in particular for crops (e.g. [14,15]). However, to our knowledge, no comprehensive investigation has been carried out on the responses of crop physiology, biochemistry or anatomy to veterinary antibiotic exposure. Significant decreases in carrot (*Daucus carota*) root length were observed after sulfamethazine treatment with 1000 μg L^{-1} and for lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*) with 10,000 μg L^{-1} [16]. A previous study [17] showed that the antibiotic sulfadimethoxine was able to alter the root morphology of willow (*Salix fragilis* L.) plants grown hydroponically. Recently, Baran et al. [8]

Abbreviations: SA, sulfonamide; SDM, sulfadimethoxine; SZ, sulfadiazine; fm, fresh mass.

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highlighted that antibiotics, such as sulfonamides, have a very low toxicity to higher organisms like vertebrates, but a much higher one for microorganisms, algae and certain plants.

Since root architecture is closely linked with the plant acquisition of water and nutrients from the soil [18], we investigated the effects of two antibiotics on growth parameters, morphological structure and membrane integrity of barley roots, with the aim of scrutinizing more in depth the effects of veterinary antibiotics on this important crop plant.

For this reason, plants were grown in a nutrient solution containing 40 μM of sulfadimethoxine (SDM) and sulfamethazine (SZ), two sulfonamide antibiotics (SAs) extensively exploited for routine mass treatment of farm animals [5]. A dose of 40 μM, corresponding to 12 and 11 mg L^{-1} of SDM and SZ respectively, was chosen as it falls in the range of the SA concentration, previously mentioned, mainly found in arable lands and manure. In addition, to have a wider picture of the effects of SAs on barley plants, the structural organization and also the photosynthetic pigment levels were compared in leaves of control plants and plants treated with the two antibiotics.

2. Results and discussion

2.1. Roots

2.1.1. Total biomass

The total plant biomass (Fig. 1a) of both control plants and plants selected for the treatment with the two SAs revealed values of approximately 0.2 g fm each, before starting the experiment. Even during the period of SA exposure the total biomass of the three treatment groups showed trends towards constant growth. Masses of the samples treated with SDM and SZ showed a slight decrease compared to controls at the second analysis point, at 8 d after the beginning of the exposure, and the decrease persisted through the whole period of treatment. However, these differences were not statistically significant. Moreover, the biomass of the epigeal apparatus of barley plants was not different among the three groups (approximately 0.45 g per plant at the end of the experiment), confirming that the root biomass was also comparable. Higher SDM concentrations, from 50 to 450 mg L^{-1}, induced strong inhibitions of the fresh mass production of *Azolla filiculoides* Lam., *Lemna minor* L. and *Pistia stratiotes* L. grown in a nutrient solution [19].

Even though the SA treatment did not affect the total biomass of barley roots, important alterations of the whole root apparatus structure were found, with shorter primary root formation (Fig. 1 b) and a less developed root area (Fig. 1c) in comparison to control plants. This phenomenon was visible in treated plants with both the antibiotics, with a much more pronounced effect after SZ than SDM exposure. In particular, looking at the total root lengths (i.e., primary and secondary roots together) (Fig. 1b), significant differences ($p < 0.05$) appeared at the 8 d from the beginning of the exposure and, at the end of the experimental time, plants exposed to SDM and SZ showed a total root length equal to 76% and 49% of the control group, respectively. Similarly, the root area values (Fig. 1c) were roughly consistent with those concerning the total lengths, showing a significant ($p < 0.05$) and much more visible inhibitory effect exercised by SZ. Therefore, it can be deduced from these results that the two SAs employed are able to cause a decrease in the root elongation rate, with a less compromising effect generated by SDM than by SZ.

Root elongation is often considered to be a primary effect measure in plant toxicity tests because the roots are the direct point of contact with the toxic medium and contaminants may enter the plant through them [20]. Evidence highlighted by the present study

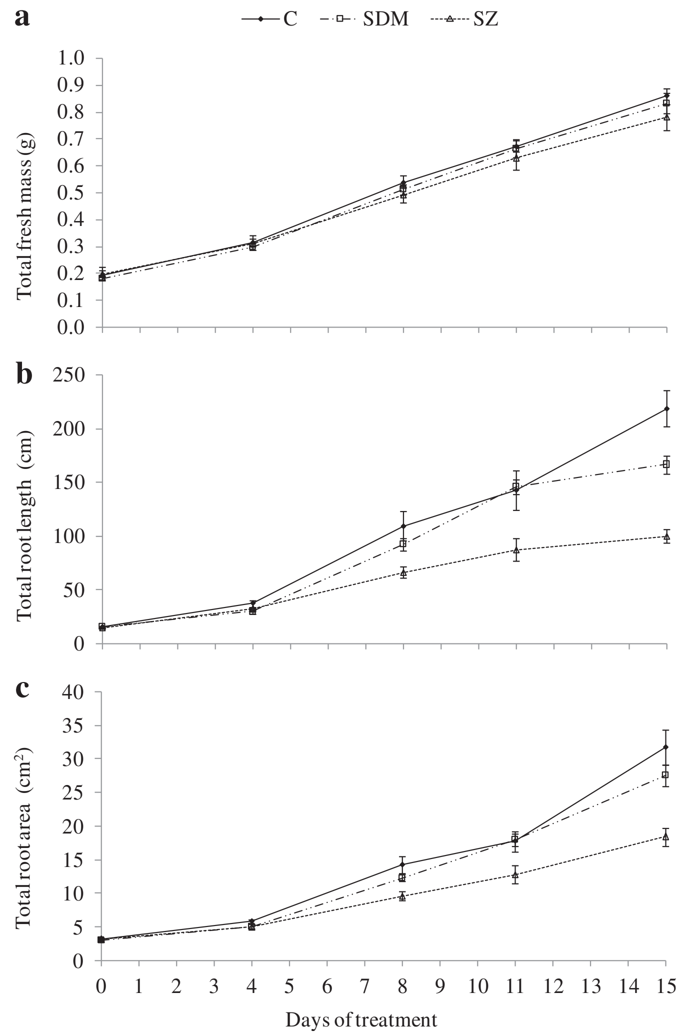

Fig. 1. Total fresh mass (a), total root length (b) and total root area (c) of barley plants exposed to 0 (C) or 40 μM of SDM or SZ at different times during the treatment period.

supports the use of root development analysis in surveying the plant response to pollutants in short-duration experiments [17,20].

Among the more evident SA effects on the plants used in this study there is the contaminant action on the overall root architecture. In fact, after 15 d from the beginning of the experiment, control plants showed very long and thin primary roots, while lateral ones were visible about 10 cm behind the root tips (Fig. 2a). Conversely, SDM and SZ treatments led to much shorter primary roots and numerous lateral ones a few mm from the apexes (Fig. 2b, c). Shorter roots were also noticed in *L. minor* L. plants exposed for 5 weeks to a flumequin antibiotic at concentrations from 50 to 1000 μg L^{-1} [21]. The serious consequence on root elongation induced by both SZ and SDM might be explained by the inhibitory effect of these compounds on folic acid synthesis. In fact, sulfonamides are structural analogues of *p*-aminobenzoic acid (*p*ABA), thus operating as competitive inhibitors of dihydropteroate synthase, which is involved in folate biosynthesis in both microorganisms and plants [22,23]. This vitamin is essential for a set of reactions that involve the transfer of single-carbon units (C1 metabolism) and then for many crucial metabolic processes, including the biosynthesis of purine and pyrimidine bases of DNA and RNA [24,25]. However, the shortening of the primary roots in our treated plants was accompanied by the development of a large