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Interspecific differences in growth response and tolerance to the antibiotic sulfadiazine in ten clonal wetland plants in South China

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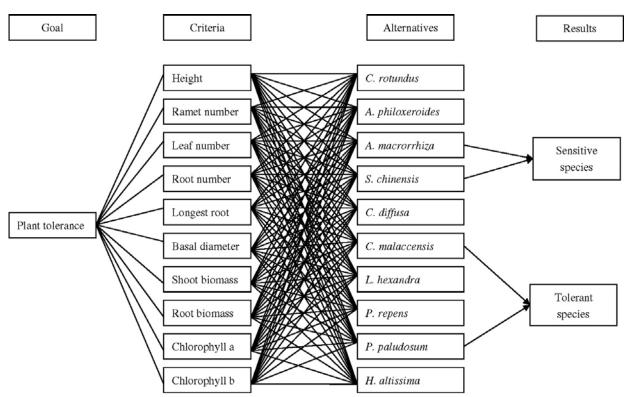
HIGHLIGHTS

- *Alocasia macrorrhiza* and *Saururus chinensis* were susceptible to sulfadiazine,
- while *Panicum paludosum* and *Cyperus malaccensis* var. *brevifolius* were tolerant.
- Ramet number was not sensitive to sulfadiazine 10 mg kg^{-1}.
- The fast shoot growth rate may contribute to the high tolerance to sulfadiazine.

GRAPHICAL ABSTRACT



Schematic diagram for selection of sulfadiazine tolerant species

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ABSTRACT

Pollution caused by residual antibiotics is a worldwide environmental issue. Antibiotic residues often occur in aquatic ecosystems, posing threats to the health of aquatic organisms. The effects of antibiotic residues on the growth of crop plants and on human health are reasonably well known. However, less is known about antibiotic effects on wetland plants. Therefore, we studied the response and tolerance of ten clonal wetland plants grown in soil spiked with sulfadiazine at 10 mg kg^{-1} (an environmentally relevant concentration) and 100 mg kg^{-1}. At 10 mg kg^{-1}, ramet number was the least affected trait, while root number was the most affected among plant species. Plant shoot and total biomass were reduced in all species except in *Cyperus malaccensis* var. *brevifolius* and *Panicum repens*. Chlorophyll content was reduced in *Alocasia macrorrhiza*, *Saururus chinensis*, and *Commelina diffusa*. In general, *Panicum paludosum* and *C. malaccensis* var. *brevifolius* showed the least reduction of growth parameters, whereas growth of both *A. macrorrhiza* and *S. chinensis* was severely reduced. At 100 mg kg^{-1}, negative responses occurred in all species. Comprehensive tolerance analysis revealed that *P. paludosum* and *C. malaccensis* var. *brevifolius* were the species most resistant to sulfadiazine. These species are potential candidates for sulfadiazine polluted wetland restoration. *A. macrorrhiza* and *S. chinensis* were the most susceptible species and they

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should be protected from sulfadiazine pollution. Relative plant shoot biomass and height were the most useful indicators for evaluating plant tolerance to sulfadiazine. Plant tolerance to sulfadiazine was associated with the differences of plants in height and shoot biomass.

1. Introduction

Antibiotics are widely used for therapeutic treatment of infectious diseases and also for disease prevention and growth promotion. Most antimicrobial compounds are poorly absorbed by animal intestinal tracts and large amounts are excreted intact and enter the environment (Kumar et al., 2005). Aquatic ecosystems are prone to antibiotic pollution via (1) direct input; examples include antibiotics administered to fish or shrimp in aquaculture farms as medicated feed additives added directly to the water (Le Bris, 1996); (2) surface runoff, in which antibiotics are transported to water from contaminated sources such as animal farms and fields fertilized with animal manure (Campagnolo et al., 2002; Tong et al., 2009); and (3) urban wastewater treatment plants, where inadequately treated antibiotics are discharged as effluent into lakes or rivers (Michael et al., 2013). Antibiotics are frequently detected in aquatic ecosystems around the world (Kümmerer, 2009).

Sulfonamides are a class of antibacterial agents intensively used in animal production. The concentration of sulfonamides in aquatic ecosystems, including man-made aquatic ecosystems, such as fish and shrimp farms, ranges from μg L^{-1} to mg L^{-1} in waters (Hoang et al., 2011; Kümmerer, 2009), and μg kg^{-1} to mg kg^{-1} in soils or sediments (Hoang et al., 2011; Kemper, 2008). Most antibiotics entering aquatic systems are absorbed into sediments, where they are either degraded (Lai et al., 1995) or slowly leaching down into the water column (Smith and Samuelsen, 1996). The elevated concentration of some antibiotics found in surface water is toxic to organisms, such as cyanobacterium exposed to ciprofloxacin (Ebert et al., 2011), green alga to tetracycline (González-Pleiter et al., 2013) and the macrophyte *Lemna minor* to ciprofloxacin (Ebert et al., 2011). Conkle and White (2012) found that tetracycline and sulfadiazine, at environmentally relevant concentrations, affected wetland soil respiration. Finally, the induced antibiotic resistance of microbes in antibiotic contaminated matrices poses a great threat to human health (Cabello, 2006).

Phytoremediation is a potential way to purify antibiotic contaminated waters and soils and thus reduce the risk of antibiotic resistance (Gujarathi et al., 2005). Plants can uptake, transport or degrade antibiotics (Gujarathi et al., 2005; Michelini et al., 2012; Migliore et al., 1995, 1996, 1997, 2000, 2003; Pramer, 1953; Thi et al., 2012; Thuy et al., 2013). The mechanisms for plant removal of antibiotics involve: (1) uptake, transport, accumulation, and degradation, with plant uptake being only a small fraction of the total amount of antibiotics in the environment (Dolliver et al., 2007; Michelini et al., 2012) and (2) rhizosphere degradation such as antibiotic modification by root exudates (Gujarathi et al., 2005) or indirect effects of the rhizosphere on microorganisms that are able to degrade antibiotics in sediments (Maki et al., 2006).

Phytoremediation of antibiotic polluted matrices involves proper selection of antibiotic tolerant plants. Antibiotic effects have been documented on many plants, but most of these studies focused on antibiotics effects on crops in terms of production and food security (Boxall et al., 2006; Eggen et al., 2011; Liu et al., 2009). Less attention has been focused on the phytoremediation potential of wetland plants. Common reed (*Phragmites australis*) exposed to an antibiotic mixture of ciprofloxacin HCl, oxytetracycline HCl, and sulfamethazine, had reduced root activity, and chlorophyll content when the antibiotic concentration was 10 μg L^{-1}, a level often found in aquaculture wastewater (Liu et al., 2013a). This indicates that some wetland plants might suffer from antibiotic toxicity during phytoremediation. Many antibiotics can adversely affect plant growth, the extent of which depends on antibiotic concentration and plant species (Carvalho et al., 2014). The adverse effects are associated with direct uptake of the antibiotics. Similar phytotoxicity is induced using soil matrices, even though antibiotic bioavailability in soil is generally lower than in aqueous solutions (Carvalho et al., 2014). In soils, phytotoxicity may be associated with both direct plant uptake of the antibiotics and interactions between plants and rhizosphere microbes (Carvalho et al., 2014; Grassi et al., 2013). There are important relationships between plants and rhizosphere microbes (Berg, 2009; Whipps, 2001). Antibiotics may affect plant development indirectly by influencing the structure and function of the rhizosphere microbial community (Reichel et al., 2015). Although it is unclear which effects play a major role in affecting plant growth under antibiotic treatment, it is reasonable to use sediments as a matrix for studying the toxic effects, as most antibiotics entering aquatic environment are absorbed into sediments (Conkle et al., 2010; Liu et al., 2013b).

Clonal plants are those that produce one or more genetically identical descendants (ramets) through clonal growth (Dong, 2011). Clonal wetland plants are an integrated part of the wetland plant community. In China, clonal plants account for 66.79% of all the wetland plant species, and have an importance value (density + frequency + coverage) of 69.55% (Song and Dong, 2002). The unique traits of clonal plants, such as clonal growth, clonal plasticity, and clonal integration, allow good adaptability to their environment, and make these plants potential candidates for ecosystem engineering (Dong, 2011). Several clonal wetland plant species have been used, in polluted or degraded wetlands, as components of remediation or restoration (Rai, 2008; Weis and Weis, 2004). Till now, a lot of work has been done on using wetland plants for phytoremediation of heavy metal contaminated wastewater (Chandra and Yadav, 2010, 2011; Yadav and Chandra, 2011). However, the toxic effects of antibiotics to clonal wetland plants, and plant antibiotic tolerance have not been investigated systematically. The goal of this study was to assess toxic effects of sulfadiazine on ten clonal wetland plants found in South China. We then evaluated plant tolerance and discuss possible tolerance mechanisms.

2. Materials and methods

2.1. Materials and experiment design

The experiment was conducted in a research greenhouse facility in Guangdong Academy of Agriculture Science (GDAAS), Guangzhou, China (E 23°09′02.35″; N 113°21′07.98″). The experiment site is located in a subtropical monsoon zone with an annual precipitation exceeding 1600 mm, annual mean temperature ~ 21 °C, and mean relative humidity 77%. Seedlings of *Cyperus rotundus* (CR), *Alocasia macrorrhiza* (AM), *Saururus chinensis* (SC), *Cyperus malaccensis* var. *brevifolius* (CM), *Leersia hexandra* (LH), *Panicum repens* (PR), *Hemarthria altissima* (HA) or cuttings of *Alternanthera philoxeroides* (AP), *Commelina diffusa* (CD), and *Panicum paludosum* (PP) were obtained from a native plant nursery located on the experiment farm of GDAAS, where several plant species were grown for experimental and wetland restoration proposes. Except for AP, all are native plant species common in South China. AP is an invasive plant originating in South America. All species grow well in the typical red soil and climate of South China.