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Maize root culture as a model system for studying azoxystrobin biotransformation in plants

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HIGHLIGHTS

- Fast and easy-to-handle maize root model to study xenobiotic metabolism in plants.
- Azoxystrobin metabolism in the model comparable to that in a real plant of another species.
- Mass spectrometric confirmation of azoxystrobin metabolites.

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ABSTRACT

Hairy roots induced by *Agrobacterium rhizogenes* are well established models to study the metabolism of xenobiotics in plants for phytoremediation purposes. However, the model requires special skills and resources for growing and is a time-consuming process. The roots induction process alters the genetic construct of a plant and is known to express genes that are normally absent from the non-transgenic plants. In this study, we propose and establish a non-transgenic maize root model to study xenobiotic metabolism in plants for phytoremediation purpose using azoxystrobin as a xenobiotic compound. Maize roots were grown aseptically in Murashige and Skoog medium for two weeks and were incubated in 100 μM azoxystrobin solution. Azoxystrobin was taken up by the roots to the highest concentration within 15 min of treatment and its phase I metabolites were also detected at the same time. Conjugated metabolites of azoxystrobin were detected and their identities were confirmed by enzymatic and mass spectrometric methods. Further, azoxystrobin metabolites identified in maize root culture were compared against azoxystrobin metabolites in azoxystrobin sprayed lettuce grown in green house. A very close similarity between metabolites identified in maize root culture and lettuce plant was obtained. The results from this study establish that non-transgenic maize roots can be used for xenobiotic metabolism studies instead of genetically transformed hairy roots due to the ease of growing and handling.

1. Introduction

Plants have defensive mechanism by which foreign chemicals, called xenobiotics, are metabolized through a cascade of enzymatic reactions broadly categorized as Phase I and phase II metabolism (Sandermann, 1994; Huber et al., 2012; Chen et al., 2016). Ideally, a xenobiotic compound is metabolized to its more hydrophilic form through phase I and phase II metabolism and finally sequestered in vacuoles or cell walls. Phase I reactions are initiated by cytochrome P450 complex leading to oxidation, reduction or hydroxylation of xenobiotics. Typically, a phase II reaction in plants involves conjugation of phase I metabolites with glucose, glutathione or malonyl-glucose. Glucosylation is catalyzed by glucosyl-transferase enzyme. Further, a glucoside conjugate can subsequently get converted to malonyl glucoside conjugate due to the action of malonyl-CoA transferase (Sandermann Jr., 1992). The information on xenobiotic metabolites in plants is required during new pesticide active ingredient development (Mullins, 1993) or for human and environmental safety assessment (E.F.S.A, 2012).

The metabolic fate of xenobiotics in plants have been studied using model systems (Van Eerd et al., 2003; Myung et al., 2013) due to the associated advantages of rapid growth and control of photo

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or microbial degradation (Chen et al., 2016), the factors which could be difficult to achieve while using real plants. Hairy roots induced by *Agrobacterium rhizogenes* are widely used for elucidation of xenobiotic metabolism in plants (Cherian and Oliveira, 2005; Huber et al., 2009, 2012; Aken et al., 2010; Macherius et al., 2014; Chen et al., 2016). However, induction of hairy roots by *A. rhizogenes* can alter the genetic construct of plant tissues (Nishikawa and Ishimaru, 1997) and may lead to altered metabolic activity of the tissues (Ciau-Uitz et al., 1994; Alikaridis et al., 2000). Further, the genetic transformation of plant tissues requires specialized skills and takes a long time (at least four weeks) and needs repeated subcultivation (Nepovím et al., 2004). Thus, it would not be ideal for laboratories with limited or no experience working with bacterial cultures and genetic transformation. So, a model system other than hairy root that can produce results comparable to xenobiotic metabolism in real plants is desirable.

Maize plant contains a variety of xenobiotic detoxification enzymes in its tissues (Sari-Gorla et al., 1993). A higher production of detoxification enzymes in roots than in shoots was observed in maize during events of xenobiotic stress caused by herbicides and pesticides (Dixon et al., 1997). Thus, maize root could be a potential tissue for developing into a model system to study xenobiotic metabolism. Herein, we hypothesize that using a maize root model, we can mimic the xenobiotic metabolism in plants in a very short duration while controlling factors like photo or microbial transformation of xenobiotics.

The main aim of this study was to develop a maize root culture as a model system to study xenobiotic biotransformation in plants. Another aim was to use quadrupole-linear ion trap mass spectrometry and data dependent acquisition to identify xenobiotic metabolites in plants using azoxystrobin as a model compound. We developed maize root culture and used it to study metabolism of azoxystrobin and compared the metabolism of azoxystrobin in maize root culture with that in lettuce sprayed with azoxystrobin. Mass spectra for azoxystrobin metabolites from maize roots and lettuce were acquired and the identities of some conjugated azoxystrobin metabolites were confirmed by performing enzymatic hydrolyses.

2. Materials and methods

2.1. Chemicals and solvents

Azoxystrobin (Az) (99.4%), azoxystrobin metabolite 2-hydroxybenzonitrile (2HBzN), almond derived β-D glucosidase and phytagel were purchased from Sigma-Aldrich (Copenhagen, Denmark). Other azoxystrobin metabolites 4-(2-cyanophenoxy)-6-hydroxypyrimidine (Az-pyOH) and 2-{6-(2-cyanophenoxy)pyrimidin-4-yloxy}benzoic acid (Az-benzoic) were purchased from HPC Standards GmbH (Cunnersdorf, Germany). Azoxystrobin metabolite azoxystrobin free acid (AzFA) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile and methanol (TOF/MS grade) was obtained from Fisher Chemical (Roskilde, Denmark). A Milli-Q system (Millipore, MA, USA) delivered ultrapure Milli-Q water used for extraction and analysis. Murashige and Skoog (M&S) medium containing vitamins was purchased from Duchefa Biochemie (Harlem, The Netherlands). Sucrose (≥99.5%) was obtained from Sigma.

2.2. Maize root culture

Maize seeds, cultivar Kaspian, was obtained from KWS Scandinavia (Vejle, Denmark). The seeds were surface sterilized by rinsing with 70% ethanol for 1 min, shaking in 1.5% sodium hypochlorite for 20 min, followed by repeated washing with sterilized Milli-Q water, and finally soaking in sterilized water for 5 min. The sterilized seeds were transferred into petri plates (10–12 seeds per plate) containing full-strength M&S medium (4.4 g/L), phytagel (3 g/L) and sucrose (20 g/L) with pH maintained at 5.8 (Fig. 1a). Every aseptic activity was done on a sterile bench. The plates were left for germination at 23 °C under light/darkness for 16 h/8 h for seven days (Fig. 1b). The roots were detached aseptically from seedlings after seven days of germination (Fig. 1c and d) and transferred into Erlenmeyer flasks (Fig. 1e) that were autoclaved with half-strength M&S liquid medium (2.2 g/L) at pH 5.8. The flasks were covered with aluminum foil to stop light from entering into the flasks and were shaken in a Certomat S II rotatory shaker (Sartorius, Taastrup, Denmark) at 100 rpm at 25 °C for seven days. The maize root culture was then ready for uptake and metabolism study (Fig. 1f).

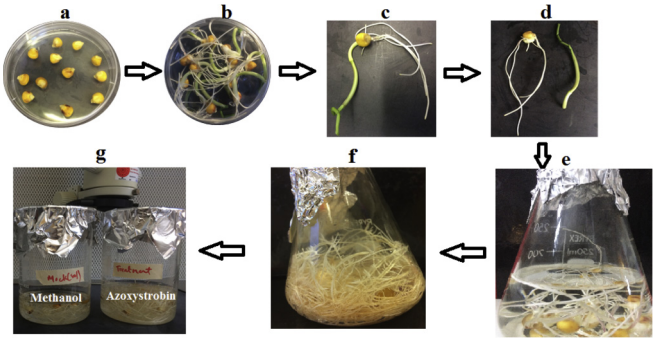

Fig. 1. Pictorial representation of maize root germination, growth and azoxystrobin metabolism in the order: a) surface sterilized maize seeds on full strength M&S solid medium in a petri plate b) germination of maize after one week in full strength M&S solid medium c) a single maize seedling with intact shoot and root d) a maize seedling with shoot detached e) maize roots suspended in half-strength M&S medium in an Erlenmeyer flaks f) maize root culture after growth of maize roots in half-strength M&S medium for 1 week g) maize root culture during azoxystrobin uptake and metabolism experiment.

2.3. Lettuce cultivation and spraying

The metabolism of a xenobiotic in a model needs to be very similar to that in real plants in order to be a viable model system (Doran, 2009). To establish the suitability of using the maize root model to study azoxystrobin biotransformation in plants, we compared the biotransformation of azoxystrobin in maize roots against that in a green-house grown lettuce plant. In this way, a comparison between results obtained from the model system could be compared to that in a plant of different species, in this case lettuce.

Romaine lettuce seeds of cultivar Quintus RZ were obtained from Rijk Zwann (De Lier, The Netherlands) and grown in a greenhouse at the Research Center Flakkebjerg, Aarhus University. Lettuce seedlings were planted in a 5-L pot, one plant a pot, filled with soil, peat and sand (2.1:1 w/w). The control and treatment groups were grown in separate tables and watered automatically 30 min daily. The plants were sprayed when they were one month old, using a pot sprayer. The sprayer was calibrated to deliver 517 L/ha spray volume with a supplier recommended dose of 1 L/ha Amistar®. The sprayer was set at a boom speed of 2.3 km/h and the spray pressure of 3.5 bar. Spraying was done through a nozzle (size O2) that was available from Hardi (Taastrup, Denmark). Two sprayed plants were randomly chosen and harvested one week after spraying, placed in plastic bags and stored at −20 °C, freeze dried in Drywinner 6 85 freeze drier from Holm & Halby (Brøndby, Denmark) and used for analysis. An unsprayed plant was also harvested and treated similarly.