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Metabolomics reveals defensive mechanisms adapted by maize on exposure to high molecular weight polycyclic aromatic hydrocarbons

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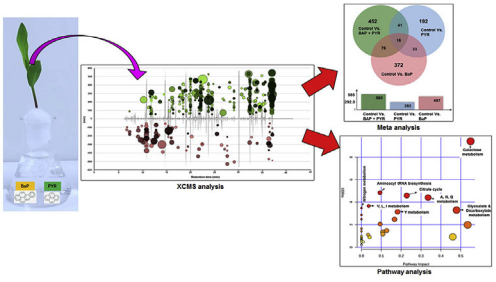
HIGHLIGHTS

- Assessed the impact of PAHs on regulation of maize metabolism.
- Metabolite response was significant between control, individual & PAHs mixtures.
- Galactose & aminoacyl tRNA biosynthesis pathways were greatly affected by PAHs.
- Provide better insights on overall stress response of maize towards PAHs.
- Adaptive defensive mechanisms actuated by PAHs exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons are an important group of persistent organic pollutants. Using plants to remediate PAHs has been recognized as a cost-effective and environmentally friendly technique. However, the overall impact of PAHs on the regulation of plant metabolism has not yet been explored. In this study, we analyzed the alteration in the maize (*Zea mays* L.) metabolome on exposure to high molecular weight PAHs such as benzo[*a*]pyrene (BaP) and pyrene (PYR) in a hydroponic medium, individually and as a mixture (BaP + PYR) using GC-MS. The differences in the metabolites were analyzed using XCMS (an acronym for various forms (X) of chromatography-mass spectrometry), an online-based data analysis tool. A significant variation in metabolites was observed between treatment groups and the unspiked control group. The univariate, multivariate and pathway impact analysis showed there were more significant alterations in metabolic profiles between individual PAHs and the mixture of BaP and PYR. The marked changes in the metabolites of galactose metabolism and aminoacyl tRNA biosynthesis in PAHs treated maize leaves exhibit the adaptive defensive mechanisms for individual and PAHs mixture. Therefore, the metabolomics approach is essential for an understanding of the complex biochemical

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responses of plants to PAHs contaminants. This knowledge will shed new light in the field of phytoremediation, bio-monitoring, and environmental risk assessment.

1. Introduction

Plants produce a more extensive range of metabolites than any other group of organisms, and thus metabolomics has a correspondingly higher importance in plant science (Kawamoto et al., 2010). Hence non-targeted metabolomics and unbiased analysis were employed for high throughput study of the metabolites in plants (Hill and Roessner, 2014). Currently, there are numerous techniques available to investigate the metabolomic profile in diverse biological systems, each with different degree of advantages and their choice depends on the purpose of the analysis (Fiehn, 2002). Among the various methods, gas chromatography (GC-MS) is widely used for determining the plant metabolites with high reproducibility and sharp separation (Lisec et al., 2006).

Recently, the use of untargeted metabolomics in environmental science has received much attention and has remained as a field of interest among the scientific community for the development of pollutant exposure/specific biomarkers, and the assessment of the risk of the organic and inorganic contaminants to living organisms (Jones et al., 2014; Carrizo et al., 2017). Among organic pollutants, the PAHs are ubiquitous due to their hydrophobic and recalcitrant nature. PAHs are formed naturally and by human activities such as fossil fuel burning and vehicle exhausts (Abdel-Shafy and Mansour, 2016). Despite their hydrophobicity, there are many reports on the occurrence of these compounds on the surface and groundwater bodies (Fähnrich et al., 2002; Zhang et al., 2012).

Among the PAHs, Benzo(*a*)pyrene, a high molecular weight PAH, is most extensively studied because of its ability to cause mutagenic, carcinogenic and teratogenic effects in living organisms (Samanta et al., 2002). PAHs in general are a class of aromatic hydrocarbon pollutants capable of causing cancer and mutations in animals including human beings. There are many strategies developed to mitigate PAHs pollution around the world (Kuppusamy et al., 2017). Among them, phytoremediation - use of plant species to remediate contamination, is considered as one of the best remediation strategies for the remediation of environments contaminated with PAHs. The plants with C4 photosystems especially maize was reported with high efficiency to degrade PAHs in contaminated soil (Zamani et al., 2016; Sivaram et al., 2018b). However, the application of untargeted metabolomics to study the response of maize and changes in their metabolites to PAHs exposure has not yet been investigated.

To address the knowledge gap, a differential untargeted metabolic profiling study was conducted with maize exposed to high molecular weight PAHs; BaP, and PYR alone and as a mixture (BaP+PYR). Derivatization and analysis of total metabolites from the maize leaf samples was carried out through GC-MS, with the aim to unravel the role of PAHs in inducing the metabolic alterations in maize seedlings grown in Hoagland's solution. To the best of our knowledge, this is the first report of maize metabolomic profiling exposed to PAHs in Hoagland's medium.

2. Materials and methods

2.1. Chemicals

Analytical grade BaP and PYR were purchased from Sigma-Aldrich (Sydney, Australia). The stock solutions were prepared in dimethylformamide (DMF) as this solvent was considered as a solvent of choice for toxicity studies (Subashchandrabose et al., 2015).

2.2. PAHs spiking

The Hoagland's solution was prepared according to Hoagland and Arnon (1950). The stock solution of BaP and PYR were spiked into 50 mL sterile Hoagland's solution (10 mg L^{-1}) in Erlenmeyer's flask (250 mL). The selected concentration was in the range of the concentration found for these compounds previously recorded from the natural environment at heavily polluted sites (Fähnrich et al., 2002). The concentration of BaP and PYR was determined using High-Performance Liquid Chromatography (HPLC) with −UV Vis detector; the separation was carried out in a Zorbex Eclipse Column XDB-C18 as described in our previous work (Sivaram et al., 2018b). The final measured concentrations of BaP and PYR in Hoagland's solution on the day one were 9.8 and 9.7 mg L^{-1}, respectively. The treatments for metabolomic profiling comprised of unspiked control, BaP, PYR, and BaP + PYR mixture and each treatment were replicated thrice.

2.3. Plant growth conditions

The maize (*Zea mays*) seeds were purchased from Pacific seeds, South Australia. Surface sterilization was carried out according to Sivaram et al. (2018b) by rinsing the seeds with 95% ethanol for 10 s followed by 10% hydrogen peroxide (H_2O_2) for 3 min and 1.25% sodium hypochlorite ($NaOCl_2$) for 3–5 min and finally rinsed with sterile ultrapure water. Then the sterile seeds (10 seeds/replication) were placed on sterile Whatman no. 41 filter paper placed inside the Petri dish (150 mm) and wetted with 3 mL of sterile water. The Petri-dishes were placed in the dark at 25 °C for four days, ten replicates were maintained per treatment.

The seedlings devoid of secondary roots and a radical with the size of approximately 3 cm were selected and transferred to sterile Erlenmeyer flask (100 mL) (one seedling per flask) containing Hoagland's solution spiked with PAHs. The flasks were kept in controlled environmental conditions (of 25 ± 3/12 °C with the light and day cycle of 16/8 h) for 7 days. All the treatments and unspiked control had three replications in two sets. At the end of seven days, the seedlings were harvested, and one set of the leaves were frozen immediately with liquid nitrogen and stored at −80 °C until further analysis and another set was used to determine plant growth parameters such as root and shoot length (cm) as explained in our previous study (Sivaram et al., 2018a).

2.4. Extraction and derivatization of maize leaf samples for GC-MS analysis of polar metabolite

Extraction and derivatization of the plant samples were done in Metabolomics Australia laboratory, University of Melbourne, Australia, following the procedure by Williams et al. (2016). Briefly, about 30 mg of leaf sample was added to a cryomill tube with 0.25 mL of methanol (100%) and spiked with quantitative internal standard ($^{13}C_6$-sorbitol/$^{13}C_{15}$N-Valine in water, 0.2 mg mL^{-1}).