Characterization of Maize Cytochrome P450 Monooxygenases Induced in Response to 2,3,7,8 Tetrachlorodibenzodioxin: The Role of Indigenous Biomarkers in Environmental Monitoring of Contaminated Sites

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Short Report

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Abstract

The objective of this research is to evaluate the potential of detecting or degrading 2,3,7,8 Tetrachlorodibenzodioxin (TCDD) in soil using a native plant growing in a contaminated site by gene expression of Cytochrome P450 monooxygenases (P450s) method. A significant difference in the root length (range of value: 580.2 mm to 799.2 mm) and enzyme activity (range of value: 31.2 to 82.3 nmolmin -1 g -1 total protein) of such indigenous plant was found in 10 µg/L TCDD treatment when compared to other treatments. Thirteen- and twenty-fold levels of gene expression in CYP71C1 and CYP79A61 of the plant growing in the contaminated site were found after 10 µg/L TCDD treatment. Such indigenous plant is sensitive to the detection of such persistent organic pollutant in the field site and involves gene expression change facilitated by a plant-microbe symbiotic association.

Introduction

Cytochrome P450 monooxygenases (P450s) are an abundant anti-toxicant compound present in various organisms including microorganisms, plants and animals (Behrendorff, 2021). Many scientists discovered P450s isozymes can capable of binding oxidative compounds. Cytochrome P450s is a family of enzymes found mainly in the liver of mammalian vertebrates or in the gut of lower-class animals and plant's body. The induction of Cytochrome P450 monooxygenases has been established as a biomarker of sublethal exposure to dioxins (Nebert and Dalton, 2006).

Frear et al., (1969) firstly discovered that the involvement of P450s was found in detoxification of 3 phenyl-1-methylureas (herbicides) in cotton seedlings due to the secretion of b5 cytochrome and NADPH-cytochrome (reductase). Therefore, it was proposed an indicator of applying herbicide in the contaminated site. Although induction of cytochrome gene was used as a biomarker of exposure to dioxin-like compounds in different organisms (Rattner et al., 1989; McLemore et al., 1990; Stegeman and Hahn, 1994; Bucheli and Font, 1995; Letcher et al., 1996; Ibrahim et al., 2020), reports on the study of P450s in plant was rare. A few examples included *Arachis hypogaea* (Liu et al., 2014), *Medicago truncatula*(Carelli et al., 2011), *Arabidopsis* (Kushiro et al., 2004) however these plants are less advantageous to detect specific toxicant possibly present in the site. This is because according toGadzala-Kopciuch et al., (2004), the criteria of being a bioindicator used in the field site should high accumulating capacity, stable population, wide range of toxicant tolerance, simple identification, and sampling, abundance and wide distinction in nature. Li and Wei (2020) illustrated the family of P450 gene in maize and wheat however knowledge gap about CYPs quantitation in the plant grown in the contaminated land after exposure to 2,3,7,8 TCDD is uncleared.

According to the previous studies, 2,3,7,8 TCDD were released into the environment through incineration, electrical generation in factory and motor vehicle exhaust. Such pollutant was commonly found in different countries such as Estonia (0.001-0.002 µg/kg) (Roots et al., 2004), Swiss (0.011 µg/kg) (Schmid et al., 2005), Japan (0.043 µg/kg) (Sakurai et al., 2000), Korea (3.72 µg/kg) (Im et al., 2002), Hong Kong (109 µg/kg) (HKEPD, 2002). Exposure of dioxin to human may lead to acute and chronic health effect including dermal toxicity, immunotoxicity, endocrine disruption, carcinogenicity and reproductive disruption. Therefore, plant may contribute to detect and degrade organic pollutant directly [i.e. plant cycle may utilize nutrient together with pollutant (Roots et al., 2004)] and indirectly [i.e. altered microbial populations within the rhizosphere zone within the root (Read et al., 2008)].

There are growing evidence of the presence of P450 in some cultivated plantssuch as plant used in Chinese medicine(Ashour et al., 2017), transgenic plant (Kumar et al., 2012), cash crop (Li and Wei, 2020) but less reported was found in an indigenous plant grown in the contaminated site. The function of CYP in plant can enhance detoxification by lowering activation energy of herbicide metabolism (Morant et al., 2003; Komives and Gullner, 2005). However, the knowledge in gene quantitation for detecting and detoxifying pollutants and other toxicants is limited. Besides, Stylianou et al., (2020) discovered that indigenous plant grows naturally on the contaminated site and exhibited unique physiological characteristics in the site. Therefore, it was hypothesized that the possibility of P450 in native plant can act as a bioindicator in the contaminated land by increasing its gene expression levels induced by the presence of of persistent organic pollutant. Besides, the molecular mechanisms involving the regulation and expression of pollutant-sensation gene in plant especially cytochrome genes are largely unknown. Henceforth, the objective of this research is to evaluate the potential of detecting or degrading 2,3,7,8 TCDD in soil using native plantas a bioindicator grown in the contaminated site.

Materials And Method

Part 1: Preliminary investigation of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) toxicity to maize by seed germination test

Seed of maize (*Zea mays*) was sampled in four different farmlands(Figure 1) nearby the CheoyLee Shipyard (formerly) where severe dioxin contamination found in soil (109 µg/kg TCDD, according to the HKEPD report, 2002). The GPS reading of four locations were: A.

N22.310930 S114.055140; B. N22.312869 S114.031848; C. N22.31456 S 114.031861 and D. N22.318069 S114.053653. Non-indigenous seed of maize was purchased on the same farmland. The seedswere sterilized with 0.1% bleaching solution for 20 min, then washed with tap water before use. The methodology used in the study was referred to USEPA (1996).

In order to test maximum range of TCDD used in the experiment, a preliminary test was done by putting seeds into various concentration of TCDD (0.1,1,10,20,50 µg/L). Zero percentage of seed generation was observed at 20 µg/L and a concentration series of TCDD (0.001,0.01,0.1,1,10 µg/L)were applied to the germination glass bottles. 30 seeds were applied to each treatment. Further irrigations were applied to the bottles by Roison solution (Roison, 1969). There were five replicates for each group. Roison solution were used as control treatment. All bottles were placed in room temperature and kept in dark for germination. After 10 days, the root of seedlings was cut by sterile blazer. The length of root in all treatments were recorded. There were 2 types of seed x 6 concentrations x 5 replicates x 4 locations, yielding a total of 240 individual set up arranged under a randomized position in an incubator.

Part 2 Characterization of cytochrome enzyme activity in the plant

All root samples were homogenized to a fine powder after lyophilization. The dried powder was refluxed with 1L of deionized water for four hours. The extractant were evaporated and further lyophilized to ensure dryness. The solution for assay was referred to the method in Ashour et al., (2017) and the samples were prepared by dissolving the extractant to 1% dimethyl sulfide to give final concentration of 100 µg/ml. The extractant was mixed with 5mM luciferin-6-benzyl ether and incubated at 25ºC for 10 minutes. Then, the activation of enzyme activity was achieved by mixing reduced nicotinamide adenine dinucleotide, glucose-6-phosphate, magnesium chloride, glucose-6-phosphate dehydrogenase in 1M phosphate buffer and incubated the solution for 30 minutes. The solution was mixed with luciferin detecting agent and measured the absorbance within one minute in 535nm by UV-visible spectrophotometer.

In each location, all data was tested by one-way analysis of variance (ANOVA) and t-test (<0.05) to compare significant difference between TCDD concentration and root length and root length of indigenous and non-indigenous maize in the same treatment, respectively. A significant difference of root length of indigenous plant was found by comparing with non-indigenous plant only in the treatments of 10 µg/L of TCDD. Therefore, RNA extraction was then performed accordingly.

Part 3: Maize CYP protein content and qualitative analysis of gene expression

The total RNAs of root tips were extracted by RNeasy Plant Mini kit (Qiagen, US). Root tips were homogenized in liquid nitrogen using sterile RNase-free pestle and mortar. Powder of root tips was decanted into an RNase-free, liquid nitrogen cooled 1.5ml microcentrifuge tube. The RNA extraction procedure was according to the manufacturers (Qiagen, US) instruction. All glassware and plastic ware was treated with diethyl pyrocarbonate (DEPC) and autoclaved to make sure RNase-free. The experiment for cytochrome P450 mRNA quantitation was referred to Yengi et al., (2003).

Maize CYP79A61 [assession no.: AY072300 (gene); AY072296 (mRNA)], CYP71C1 [assession no.:X81827 (mRNA); X81828 (gene)] and internal control 18S ribosomal RNA (assession no.:AF168884) primers have been published previously (Persans et al, 2001; Irmisch et al., 2015). The primer for CYP79A61 and CYP71C1 were designed with modifications by using Primer 3 software and Blastn software (web-based). The primer sequences for CYP79A61 were 5'-TCCCAGGCTATTGGTTCTTG-3'; 5'-CCCAGCAAGTCGTCGTTATT-3'. The primer sequences for CYP71C1 were 5'-AACGAGCTGCTGTCCGAGTA-3'; 5'-GCTGCTTCGAACATGTTCACA-3'. The primer sequences for 18S ribosomal RNA were 5'-AGACGAACAACTGCGAAAGC-3'; 5'-GCCAGCGGGGTCCTATTAGT-3'. Each reaction contained 0.1µg/uL cDNA of root tips, 1x platinium PCR supermix (invitrogen), 0.5uM each specific primers, PCR-filtered water, make up to total volume 25uL. The amplification condition was 94 ºC for 5 min., followed by 35 cycles of 94 ºC for 30sec., 55 ºC for 30 sec and 72 ºC for 30sec. There is a final extension step of 72 ºC for 10min. GeneAmp 9700 PCR machine (Applied Biosystem, US) was used. The amplified product was analysed by 2% agarose gel (18S rRNA PCR) or 6% polyacrylamide gel (CYP71C1 and CYP79A61 PCRs) and visualized under UV.

Part 4: Semi-quantitation of CYP gene expression

Selected positive DNA fragment in both CYP71C1 and CYP79A61 with high band intensity was performed real time quantitative PCR in a Bio-Rad iQ5 System (Bio-Rad Company, CA, USA). Each reaction mixture contained a 1x final concentration of iQ SYBR Green supermix (Bio-rad), containing 0.2mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 50mM KCl, 20mM Tris-HCl, pH 8.4, iTaq DNA polymerase (25U/ml), 3mM $MgCl_2$, SYBR Green I, 10nM fluoresein, and stabilizers; 0.5uM of each specific (CYP71C1, CYP79A61 and 18S rRNA) forward and reverse primers. 2ul of each cDNA (equivalent to 150ng of reverse transcribed RNA) was used in each PCR and experiments were carried out in triplicates to ensure reproducibility. PCR conditions were as follows: The amplification condition was 94 ºC 5 min., followed by 60 cycles of 94 ºC for 30sec., 55 ºC for 30 sec and 72 ºC for 30sec. Melting curve analysis were conducted at end point of

each amplification step. Each quantitative PCR assay contained unknown samples, no template controls (reaction mix with no cDNA), and serially diluted concentrations of normal control cDNA , which act as a calibrator (range; 100ng to 1.56ng, by a 2-fold dilution), from which a relative standard curve was generated for relative quantitation of target cDNA in unknown samples. All data were normalized against 18S rRNA.

All data were presented as mean ± standard deviation. The expression level of CYP71C1 and CYP79A61 mRNA of root tips growing in different concentrations of dioxins were tested byone-way analysis of variance (ANOVA) followed by Dunnett test.

Results And Discussion

Regardless of the indigenous seeds in all locations, significant difference in the root length was found when comparing to the 10μg/L treatment to other concentrations (i.e. Location A: 799.2±6.61mm; Location B: 657.3±10.25mm; Location C 598.7±13.10; Location D: 580.2±8.92mm) (Figure 2) indicating that plant is sensitive to detect TCDD in the contaminated land and the findings was in line to Campanella and Claudia (2002). It may be subjected to the maize can uptake, translocate, and tolerate high concentration of organic pollutant intake from outside to its body(Zhang et al., 2017). However, the relationship between root length and TCDD concentration might be complicated since the deposition of burning waste in the site has ceased for many years.

By comparing to the indigenous and non-indigenous seed sampled in the same location exposure to the same TCDD concentration, a significant difference was found in 10 ug/L of TCDD treatment only (Figure 2). In the treatment of severe TCDD contamination, due to limited bacterial population and low carbon content, TCDD is a sole carbon source which facilitated the growth of microorganisms on the root surface. Thus, plants are capable of degrading chlorinated organics by direct and indirect action (Campanella and Claudia, 2002). Since the ability of detecting TCDD by organism is strongly influenced by environmental conditions and heredity factors,indigenous plant species can act as a bioindicator and it has clear advantage due to natural selection and plant was consequently evolved to high tolerance of adverse conditions and high TCDD concentration in an adverse conditions.

Besides, plant may secretealdoxime and aldoxime-derived compounds to protect plant body attacked from xenobiotic substances or toxicants (Perez et al., 2021). Such metabolic reaction might thus forming barrier against further toxicant attack and such phenomenon is commonly found in some higher plants such as *Prunus mume* (Yamaguchi et al., 2014), *Camelina sativa* (Zhang et al., 2020) and *Phlebodiumaureum* and *Pteridium aquilinum* (Thodberg et al., 2020). Besides, the secondary metabolite such as defense proteins and chemicals synthesized by altering gene expression in the defense reaction was possible in maize (Tovar-Sánchez et al., 2018). Therefore, indigenous maize may act as a biomarker to reflect the current situation of TCDD contamination in the field site inducing different levels of gene expression exposure to toxicant. Such changes may induce mutation and shift to an adaptive population by variation through the phenomenon of directional selection (Hoffmann and Hercus, 2000). As USEPA identified TCDD is a potential human cancerogenicsubstance (i.e. low dose intake can lead to cause acute and chronic toxicity in human body), the current findings supported to detect trace amount of TCDD without using high risk, expensive, labour-intense and time consuming approach.

Table 1 describes the cytochrome enzyme activity extracted from the root of indigenous and non-indigenous maize. By comparing to the plant in the same location treated by various TCDD concentrations, the enzyme activity was significantly elevated in all 10 μg/L TCDD concentration found in indigenous maize (i.e. Location A: 31.2±0.03 $nmolmin^{-1}$ g^{-1} total protein; Location B: 59.6±0.33 $nmolmin^{-1}$ g^{-1} total protein; Location C 39.7±0.80 $nmolmin^{-1}$ g^{-1} total protein; Location D: 82.3±1.69 $nmolmin^{-1}$ g^{-1} total protein). High activity of indigenous maize sampled in location D indicated that the plant was well-adapted to the TCDD-contaminated environment. In case of non-indigenous maize, with increasing concentration, the enzyme activity of both plants varied and fluctuated irrespective of any TCDD treatments. Interestingly, yellow-coloured biofilm was found only in the root of plant treated with 10μg/L TCDD. It was speculated that plant-microorganisms associated bearing pollutant-detecting/degrading role for eliminating toxicant induced by high concentration of TCDD (Nasr, 2019).

According to Nannipieri et al., (2012), due to the variation of external conditions in the changing environment such as variation in plant cover of soil, methods in detecting enzyme activity in plant to detect pollutants are unreliable However, the results of semiquantitative RT-PCR measurement of gene expression exhibited that plants that express significant levels of CYP71C1 and CYP79A61 during the appropriate stages of plant development (Figure 3). Except to the seed sampled in location A, a band was found in all plant samples in which a pair of PCR primers were designed to be specific for CYP71C1 and CYP79A61. The specific primers amplified the 152bp and 145bp fragments from their respective target templates but not those from the non-target template.

Semi-quantitation of gene expression using real time PCR was further studied for the root of indigenous *Z. mays*exposure to 10 ug/L of TCDD. The result exhibited gene expression of CYP71C1 and CYP79A61 increased significantly, around 13 and 20 times level of the plant

sampled in location D compared to other locations, respectively (Figure 4). Apparently, thebiofilm found in the plant's root sampled in location D exhibited an alternative xenobiotic degradation to microorganism may be subjected to the expression of cytochrome gene (i.e. specific for organic pollutant degradation) (Musilova et al., 2016; Yousaf et al., 2011)facilitated by plant-microbe symbiotic association. In addition, some accessory factors such as rhizosphere zone in the root altered the composition of water and dissolved oxygen concentration may affect the genetical change and expression of microorganisms containing pollutant-degradinggenes in the colonized rhizosphere of infected plants (Afzal et al., 2014). Such symbiotic relationship induce the changes of catabolic genes (Saibu et al., 2020), meaning that the bio-degradation pathways or enzymes altered with the goal of performing specific organic pollutant degradation such as chemoorganotrophic or chemolithotrophs heterotrophs (Ijaz et al., 2016). Therefore, the changes of gene expression of specific group of microorganismsin the root of indigenous plant may trigger the efficiency of TCDD degradation.

Surprisingly, the root of seedlings with high gene expression were heavily infected by mycorrhizas. Under an adverse condition, the possibility of secreting strigolactone (Mishra et al., 2017)from plant in response to the colonization of AM fungi leads to synthesize fungi in plant root. TCDD may mimic such plant hormone to send chemical message (Olson and Morton, 2019) to plant for TCDD accumulation. Thus, the compound induced the secretion of mycorrhizal factors such as lipochitooligosaccharidesand chitin oligosaccharides (Rush et al., 2020) to enhance the formation of arbuscular and elevated plant immune responses and restore function quickly in the contaminated environment. Consequently, beneficial gene in fungi transferred to the land plants and combined into the plant cellthrough symbiotic association.

Conclusions

There was short term, immediate and long term impact of TCDD contamination on the soil by bioaccumulation and biomagnification in the food web. This is the first report of indigenous plant grown in the TCDD contaminated site exhibited up-regulation of gene expression significantly in response to TCDD. Thus, such indigenous plant is sensitive to detect TCDD in the field site facilitated by plant-microbe symbiotic association.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Ho Man Leung and Ka Chun Sung analyzed the data and were major contributors in writing the manuscript

Patrick Ying Kit Yue and Kwai Chung Cheung provided guidance on statistical analysis

Chi Kin Au and Xiao Ling Peng provided interpretation to the data

Ken Kin Lam Yung organized the plan of research

Wai Chin Li organized the plan of research

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References

1. Afzal M, Khan QM, Sessitsch A. 2014. Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. Chemosphere 117, 232–242.
2. Ashour ML, Youssef FS, Gad HA, Wink m. 2017. Inhibition of Cytochrome P450 (CYP3A4) activity by extracts from 57 plants used in traditional chinese medicine. Pharmacognosy Magazine 50, 300-308.
3. Behrendorff JBYH. 2021. Reductive cytochrome P450 reactions and their potential role in bioremediation. Frontiers in Microbiology 12, 649273.
4. Bucheli TD, Font K. 1995. Induction of cytochrome-P450 as a biomarker for environmental contamination in aquatic ecosystems. Critical Reviews in Environmental Science and Technology 25, 201-268.
5. Campanella BF, Bock, Claudia SP. 2002. Phytoremediation to increase the degradation of PCBs and PCDD/Fs: Potential and limitations. Environmental Science and Pollution Research International 9, 73-85.
6. Carelli M, Biazzi E, Panara F, Tava A, Scaramelli L, Porceddu A, Graham N, Odoardi M, Piano E, Arcioni, May S, Scotti C, Calderini O. 2011.*Medicago truncatula* CYP716A12 is a multifunctional oxidase involved in the biosynthesis of hemolytic saponins. Plant Cell 23, 3070-3081.
7. Frear DS, Swanson HR, Tanaka FS. 1969. N-demethylation of substituted 3-(phenyl)-1-methylureas: Isolation and characterization of a microsomal mixed function oxidase from cotton. Phytochemistry 8, 2157–2169.
8. Gadzala-Kopciuch R, Berecka B, Bartoszewicz J, Buszewski B. 2004. Some considerations about bioindicators in environmental monitoring. Polish Journal of Environmental Studies 13, 453-462.
9. Hoffmann AA, Hercus MJ. 2000. Environmental stress as an evolutionary force Bioscience 50, 217-226.
10. Hong Kong Environmental Protection Department. 2002. Decommissioning of Cheoy Lee Shipyard at Penny's Bay. Environmental Impact Assessment Report. HK.
11. Ibrahim M, MacFarlane EM, Matteo G, Hoyeck MP, Rick KRC, Farokhi S, Copley CM, O'Dwyer S, Bruin JE. 2020. Functional cytochrome P450 1A enzymes are induced in mouse and human islets following pollutant exposure. Diabetologia 63, 162-178.
12. Ijaz A, Imran A, ul Haq MA, Khan QM, Afzal M. 2016. Phytoremediation: recent advances in plant-endophytic synergistic interactions. Plant and Soil 405, 179–195.
13. Im SH, Kannan K, Giesy JP, Matsuda M, Wakimoto T. 2002. Concentrations and profiles of polychlorinated dibenzo-p-dioxins and dibenzofurans in soils from Korea. Environmental Science and Technology 36, 3700-3705.
14. Irmisch S, Zeltner P, Handrick V, Gershenzon J, Köllner. 2015. The maize cytochrome P450 CYP79A61 produces phenylacetaldoxime and indole-3-acetaldoxime in heterologous systems and might contribute to plant defense and auxin formation. BMC Plant Biology 15, 128-142.
15. Komives G, Gullner G. 2005. Phase I xenobiotic metabolic systems in plants. ZeitschriftfürNaturforschung C 60, 179-185.
16. Kumar S, Jin M, Weemhoff JL. 2012. Cytochrome P450-mediated phytoremediation using transgenic plants: A need for engineered cytochrome P450 enzymes. Journal of Petroleum & Environmental Biotechnology 3, 127.
17. Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E. 2004. The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO Journal 23, 1647–1656.
18. Letcher RJ, Norstrom RJ, Lin S, Ramsay MA, Bandiera SM. 1996. Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P4501A and P4502B and chlorinated hydrocarbon contaminant levels in polar bear (*Ursus maritimus*). Toxicology and Applied Pharmacology 137, 127-140.
19. Li Y, Wei K. 2020. Comparative functional genomics analysis of cytochrome P450 gene superfamily in wheat and maize. BMC Plant Biology 20, Article number: 93.
20. Liu S, Lv Y, Wan XR, Li LM, Hu B, Li L. 2014. Cloning and expression analysis of cDNAs encoding ABA 8'-Hydroxylase in peanut plants in response to osmotic stress. PLOS One9, 97025.
21. McLemore TL, Litterst CL, Coudert BP, Liu MC, Hubbard WC, Adelberg S, Czerwinski M, Mcmanoh NA, Eggleston JC, Boyd MR. 1990. Metabolic-activation of 4-Ipomeanol in human lung, primary pulmonary carcinomas, and established human pulmonary-carcinoma

cell-lines. Journal of the National Cancer Institute 82, 1420-1426.

22. Mishra S, Upadhyay S, Shukla RK. 2017. The role of strigolactones and their potential cross-talk under hostile ecological conditions in plants. Frontiers in Physiology 7, 691.
23. Morant M, Bak S, Møller BL, Werck-Reichhart D. 2003. Plant cytochromes P450: tools for pharmacology, plant protection and phytoremediation. Current Opinion in Biotechnology 14, 151–162.
24. Musilova L, Ridl J, Polivkova M, Macek T, Uhlik O. 2016. Effects of secondary plant metabolites on microbial populations: changes in community structure and metabolic activity in contaminated environments. International Journal of Molecular Science 17, 1205.
25. Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, MasciandaroG,FornasierF, Moscatelli MC, Marinari S. 2012. Soil enzymology: classical and molecular approaches. Biology and Fertility of Soils 48, 743–762.
26. Nasr M. 2019. Environmental perspectives of plant-microbe nexus for soil and water remediation. In: Kumar V, Prasad R, Kumar M, Choudhary D. (eds) Microbiome in Plant Health and Disease. Springer, Singapore. pp 403-419.
27. Nebert DW, Dalton TP. 2006. The role of cytochrome P450 enzymes in endogenous signaling pathways and environmental carcinogenesis. Nature Reviews Cancer 6, 947–960.
28. Olson KR, Morton LW. 2019. Fate of agent orange and dioxin TCDD contaminated soils and sediments in Vietnam Hotspots. Open Journal of Soil Science 9, Article ID:90675.
29. Perez VC, Dai R, Bai B, Tomiczek B, Askey BC, Zhang Y, Rubin GM, Ding Y, Grenning A, Block AK, Kim J. 2021. Aldoximes are precursors of auxins in Arabidopsis and maize. New Phytologist 231, 1449-1461.
30. Rattner BA, Hoffman DJ, Marn CM. 1989. Use of mixed-functionoxygenases to monitor contaminant exposure in wildlife. Environmental Toxicology and Chemistry 8, 1093-1102.
31. Read J, Wevill T, Fletcher T, Deletic A. 2008.Variation among plant species in pollutant removal from stormwater in biofiltration systems. Water Research 42, 893-902.
32. Rorison IH. 1969. Ecological interferences from laboratory experiments on mineral nutrition. In Rorison IH, edited, ecological aspects of the mineral nutrition of plants. Blackwell Scientific Publications, Oxford, pp.155-175.
33. Roots O, Henkelmann B, Schramm KW. 2004. Concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in soil in the vicinity of a landfill. Chemosphere 57, 337-342.
34. Rush TA, Puech-Pagès V, Bascaules A, Jargeat P, Maillet F, Haouy A, QuyManhMaës A, Carriel CC, Khokhani D, Keller-Pearson M, Tannous J, Cope KR, Garcia K, Maeda J, Johnson C, Kleven B, Choudhury QJ, Labbé J, Swift C, O'Malley MA, Bok JW, Cottaz S, Fort S, Poinsot V, Sussman MR, Lefort C, Nett J, Keller NP, BécardG, Ané -Show JM. 2020. Lipo-chitooligosaccharides as regulatory signals of fungal growth and development. Nature Communications 11, Article number: 3897.
35. Saibu S, Adebusoye SA, Oyetibo GO. 2020. Aerobic bacterial transformation and biodegradation of dioxins: a review. Bioresources and Bioprocessing 7, Article number: 7.
36. Sakurai T, Kim JG, Suzuki N, Matsuo T, Li DQ, Yao Y, Masunaga S, Nakanishi J. 2000. Polychlorinated dibenzo-p-dioxins and dibenzofurans in sediment, soil, fish, shellfish and crab samples from Tokyo Bay area, Japan. Chemosphere 40, 627-640.
37. Schmid P, Gujer E, Zennegg M, Bucheli TD, Desaules A. 2005. Correlation of PCDD/F and PCB concentrations in soil samples from the Swiss soil monitoring network (NABO) to specific parameters of the observation sites. Chemosphere 58, 227-234.
38. Stegeman JJ and Hahn ME. 1994. Regulation of cytochrome P4501A1 in Teleosts-Sustained induction of CYP1A1 messenger-RNA, protein and catalytic activity by 2,3,7,8-tetrachlorodibenzofuran in the marine fish stenotomuschrysopz. Toxicology and Applied Pharmacology 127, 187-198.
39. Stylianou E, Pateraki C, Ladakis D, Cruz-Fernández M, Latorre-Sánchez M, Coll C, Koutinas A. 2020. Evaluation of organic fractions of municipal solid waste as renewable feedstock for succinic acid production. Biotechnological Biofuels 13, 72.
40. Thodberg S, Sørensen M, Bellucci M, Crocoll C, Bendtsen AK, Nelson DR, Motawia MS, Møller BL, Neilson EHJ. 2020. A flavin-dependent monooxygenase catalyzes the initial step in cyanogenic glycoside synthesis in ferns. Communications Biology 3, 507.
41. Tovar-Sánchez E, Cervantes-Ramírez T, Castañeda-Bautista J, Gómez-Arroyo S, Ortiz-Hernández L, Sánchez-Salinas E, Mussali-Galante P. 2018. Response of *Zea mays* to multimetal contaminated soils: a multibiomarker approach. Ecotoxicology 27, 1161–1177.
42. US Environment Protection Agency. 1996. Ecological effects test guidelines: Early seedling growth toxicity test. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.
43. Yamaguchi T, Yamamoto K, Asano Y. 2014. Identification and characterization of CYP79D16 and CYP71AN24 catalyzing the first and second steps in L-phenylalanine-derived cyanogenic glycoside biosynthesis in the Japanese apricot, *Prunus mume*Sieb. et Zucc. Plant Molecular Biology 86, 215–223.

44. Yengi LG, Xiang Q, Pan J, Scatina J, Kao J, Ball SE, Fruncillo R, Ferron G, Wolf CR. 2003. Quantitation of cytochrome P450 mRNA levels in human skin. Analytical Biochemistry 316, 103-110.
45. Yousaf S, Afzal M, Reichenauer TG, Brady CL, Sessitsch A. 2011. Hydrocarbon degradation, plant colonization and gene expression of alkane degradation genes by endophytic *Enterobacter ludwigii* strains. Environmental Pollution 159, 2675–2683.
46. Zhang D, Son YH, Dai R, Lee TG, Kim J. 2020. Aldoxime metabolism is linked to phenylpropanoid production in *Camelina sativa*. Frontiers in Plant Science 11, 17.
47. Zhang S, Yao H, Lu Y, Yu X, Wang J, Sun S, Liu M, Li D, Li YF, Zhang D. 2017. Uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals by maize from soil irrigated with wastewater. Nature Scientific Report 7, 12165.

Tables

Table 1. Cytochrome enzyme activities ($nmolmin^{-1}$ g^{-1} total protein, n=5) in the root of indigenous and non-indigenous *Zea mays*. Within each column, means with the same letter are not significant different according to Duncan's Multiple Range Test ($p<0.05$)

TCDD concentration (μg/L)	Indigenous maize				Non-indigenous maize			
	Location A	Location B	Location C	Location D	Location A	Location B	Location C	Location D
0	3.87±0.88b	2.33±0.05c	1.23±0.02b	2.66±0.06c	2.19±0.04a	3.41±0.17a	1.75±0.43a	2.48±0.31a
0.001	4.58±0.59b	2.58±0.11c	3.79±0.02b	8.12±0.08bc	1.08±0.07a	2.46±0.06a	3.40±0.19a	1.37±0.09a
0.01	5.52±0.41b	4.23±0.05c	5.77±0.07b	9.64±0.88bc	2.76±0.04a	3.77±0.05a	2.03±0.07a	2.83±0.07a
0.1	5.99±0.27b	4.81±0.16c	5.53±0.30b	13.7±1.74b	3.65±0.02a	2.61±0.03a	3.47±0.02a	2.78±0.22a
1	6.18±0.06b	7.01±0.14bc	6.29±0.27b	14.5±1.07b	3.17±0.05a	3.85±0.07a	3.43±0.11a	2.79±0.21a
10	31.2±0.03a	59.6±0.33a	39.7±0.80a	82.3±1.69a	2.52±0.12a	3.96±0.02a	3.30±0.35a	3.18±0.17a

Figures

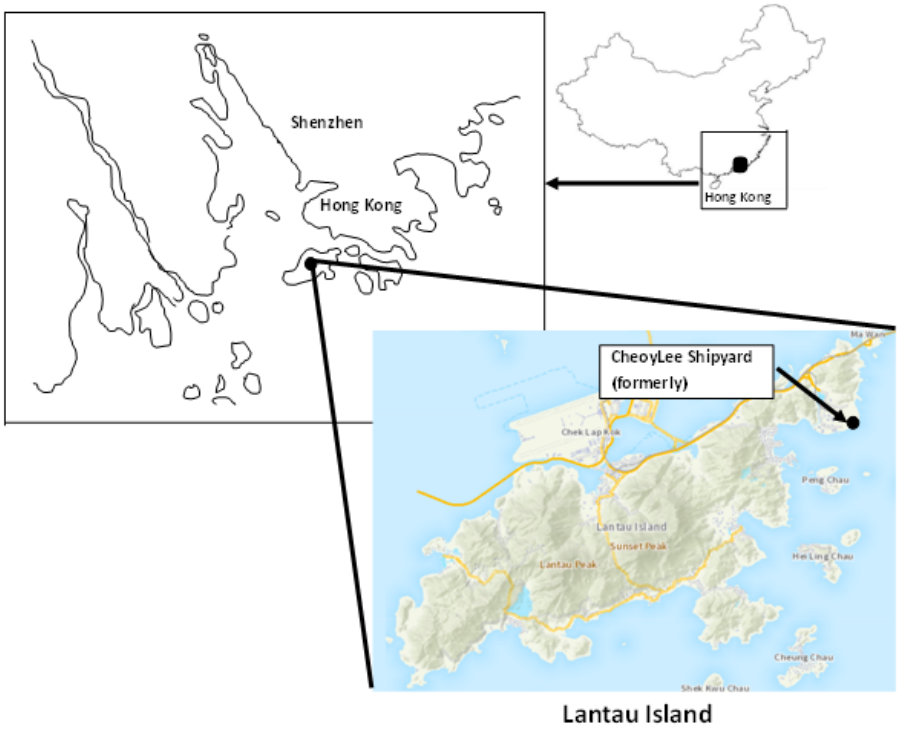

Figure 1

Sampling locations nearly CheoyLee Shipyard (formerly)

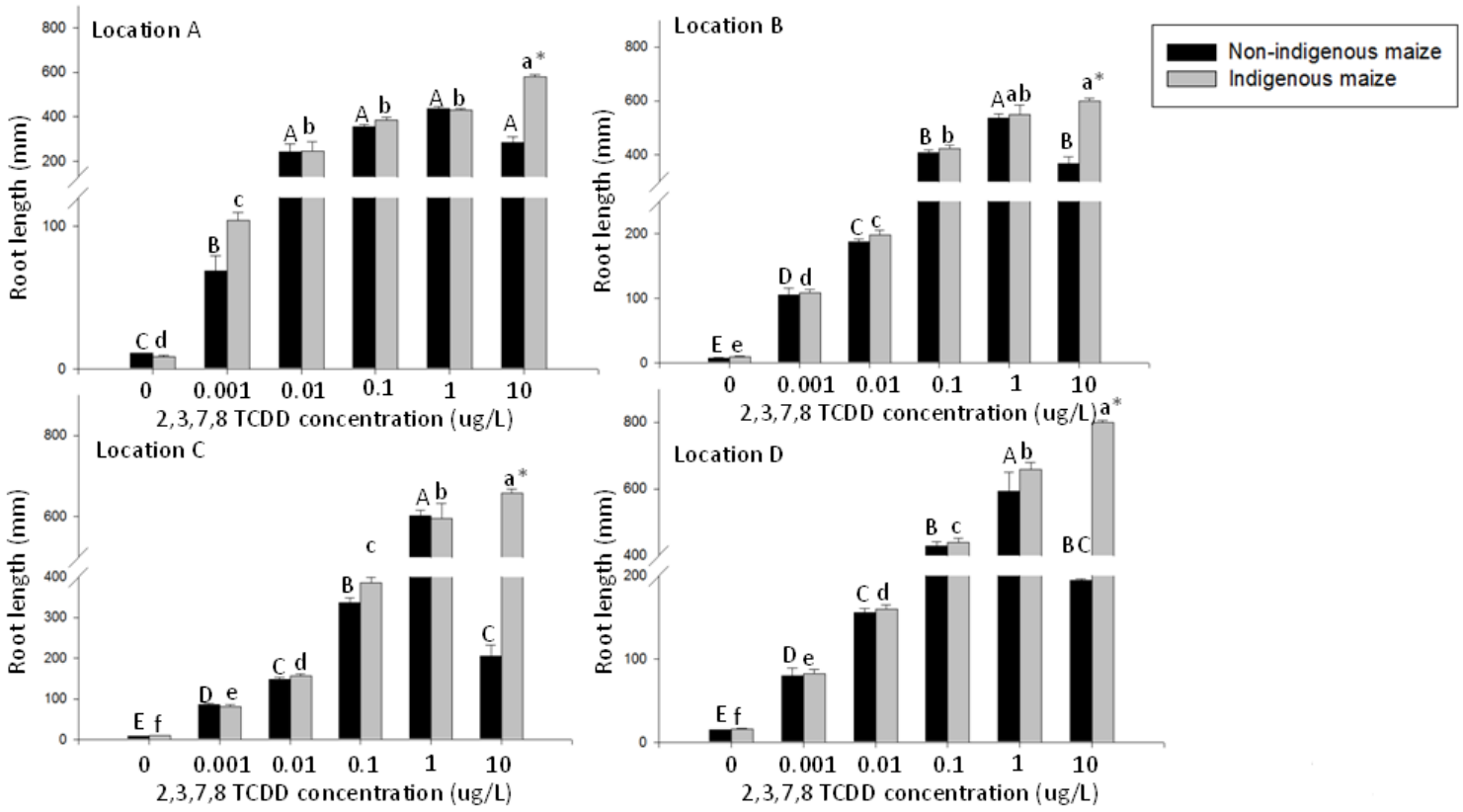

Figure 2

Root elongation of indigenous and non-indigenous *Z. mays* in different TCDD concentrations. Lower-case and upper-case lettersindicate significant difference at $p < 0.05$ according to according to Duncan's Multiple RangeTest. Significant differences between indigenous and non-indigenous plants within the same concentration estimated using t-test are indicated by asterisks.

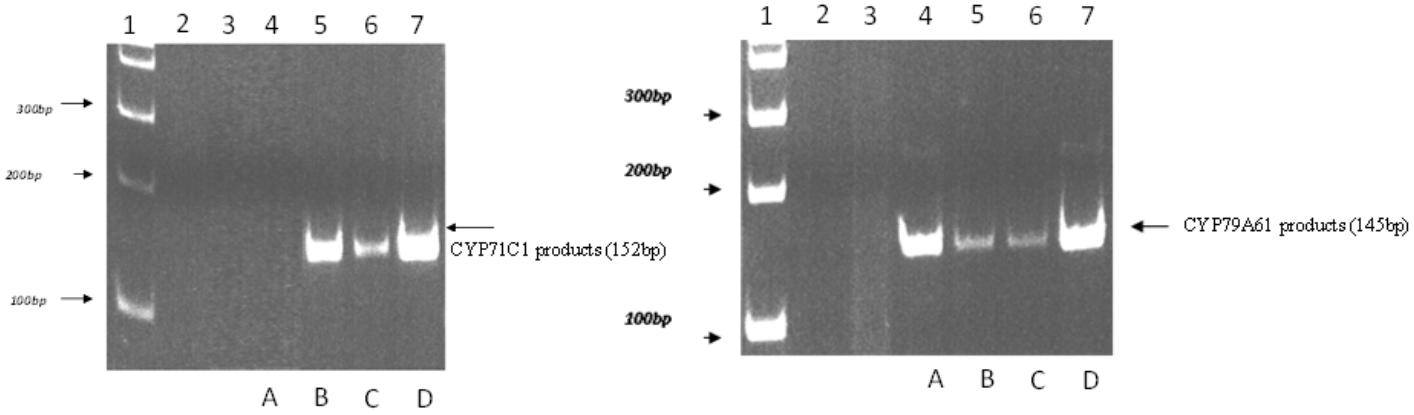

Figure 3

Gel diagram of gel electrophoresis showing RT-PCR results of CYP71C1 (left) and CYP79A61 (right). From left to right: DNA marker (Lane 1), reagent blank control (Lane 2), no RT control (Lane 3), roots sampled in locations A to D exposed to 10 µg/L TCDD (Lanes 4-7).

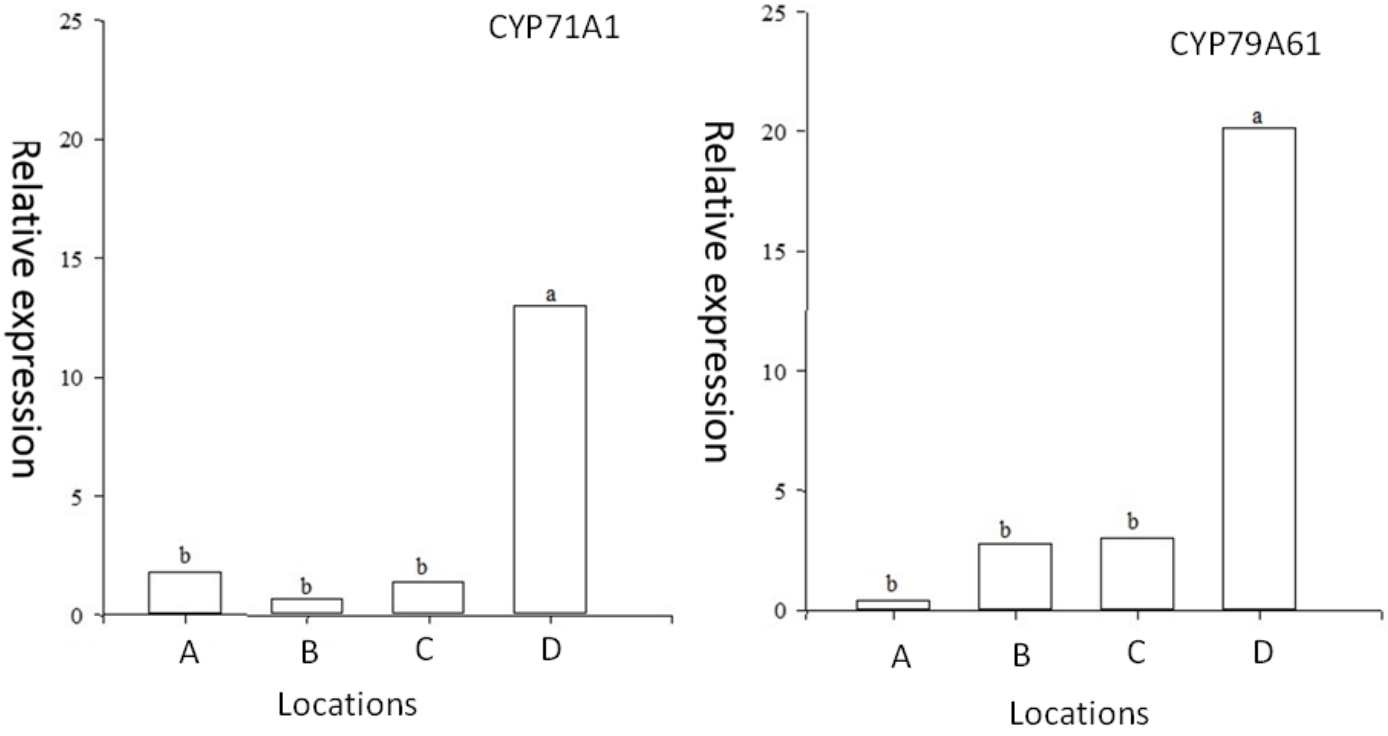

Figure 4

Relative amounts of CYP71C1 (left) mRNA and CYP79A61 (right) mRNA after exposure to 10 µg/L TCDD in root tissue. Different letters for different treatments indicate significant difference at the level of $p<0.05$ in Duncan Multiple-range test.