ISSN: 2456-8643

IDENTIFICATION OF TARGET GENES INVOLVED IN RESPONSES OF *Populus* TO POLYCHLORINATED BIPHENYLS BASED ON RNA-SEQ

Gabriela Orozco-Gutiérrez

Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias CE. Tecomán, km 35 Carretera Colima-Manzanillo. CP. 28930. Tecomán, Colima, México

Angela Contreras Mogollon

Postdoctoral of teh Universidad Politecnica de Madrid.

Centro de Biotecnología y Genómica de Plantas UPM – INIA Parque Científico y Tecnológico de la U.P.M. Campus de Montegancedo, 28223, Madrid, Spain.

Julia Quintana Gonzalez

E.S. CC. Experimentales y Tecnología de la Unviersidad Rey Juan Carlos, Calle Tulipán s/n. 28933 Móstoles, Madrid, Spain.

https://doi.org/10.35410/IJAEB.2024.5900

ABSTRACT

Steel slag and rice husk bokashi are the ameliorant that expected can decrease P-retention Phytoremediation represents an effective, low-cost, environmentally friendly alternative that improves soil properties, although there have been significant developments in the last decade. From the scientific point of life, the main challenge is to decipher the metabolic pathways involved in response to contaminants and to understand their regulation. This information is essential if we aspire to improve the natural capacities of some plant species to remediate contaminated soils. A comprehensive study of the molecular response to polychlorinated biphenyls (PCBs), a family of persistent organic pollutants of particular relevance on a global scale, was obtained. A transcriptomic approach based on RNA-seq technology has been used to identify the genes involved in the metabolism of in-plant compounds and to quantify their activation levels in different controlled situations. It is surprising that this response is virtually unknown at the molecular level, despite its great potential applied in the context of phytoremediation technology. We apply to hybrid poplar (Populus tremula x alba) crops with Aroclor 1221. And we took samples of RNA, thus generating a matrix of four elements with their corresponding controls. On the other hand, we perform functional analyzes with bioinformatic tools based on sequence comparisons and gene co-expression networks. The response of genes of particular interest was validated using qRT-PCR technology. This is the first comprehensive study of the response of a plant organism to the presence of PCBs. A considerable number of structural and regulatory genes have been found, defining new transcription factors whose expression is proportional to the concentration of pollutant in the medium or the time of exposure to it. Correlation analyzes allow us to state that the metabolic response to PCBs, including possible degrading pathways, is involved in at least fifteen transcription factors and approximately forty proteins or enzymes that are particularly induced.

Keywords: phytoremediation, RNA-seq, Populus, PCB.

1. INTRODUCTION

Phytoremediation is based on the use of plants as effective decontamination tool technology, and nonintrusive low cost. Those biological methods claim for the cleaning of the environment

especially our soil have been receiving increasing attention especially in the past two decades (Anyasi and Atagana, 2011). One of the main pollutants has been polychlorinated biphenyls (PCBs) are a family of 209 congeners on of the most dangerous is the Aroclor 12:21 (carbon: chlorine) is a commercial compound formed a mixture of PCBs (polychlorinated biphenyls). Chlorine aromatic compounds are among the worst pollutants due its toxicity, carcinogenicity, teratogenicity, and slow biodegradation widely distributed in the environment. Polychlorinated biphenyls (PCBs) are used as insulation in electrical transformers on thousands of sites, such as flame retardants in cotton clothing, and as plasticizers. Jansson and Douglas (2007) studied that poplar (*Populus* spp.) has extensive root systems and high transpiration rates, hold particular promise for Phytoremediation. Several studies suggest that organic pollutants in plants play a similar role as mammals. However, metabolic basis of degradation of PCBs by plants has not been well characterized or quantified. Here, we used Aroclor 1221 as a representative PCB to investigate the effects of growth and tolerance and the transcriptional profile of *Populus* after exposure to Aroclor 1221. An RNA-seq analysis was performed to study the molecular response to PCBS. PCBs- induced or repressed genes were identified and will be discussed.

2. MATERIALS AND METHODS

2.1 Foliar and root growth

Hybrid poplar (*Populus tremula x P. alba*) clone INRA 717. 1-B4 was used for this study. To assess vegetative growth were transferred four weeks old *in* vitro plants into 150 ml containers with MS plant growth medium (Murashige and Skoog, 1962) with Aroclor 1221 reagent (*Sigma-Aldrich*). Stock solutions for Aroclor 1221, was made up in DMSO and was incorporated immediately to the hot medium, stirring to proper homogenization. The exposure concentrations were 50 and 200 mg/l. A control was set up containing the same medium and DMSO. Primary root length and foliar dry weight was measured. Twenty seedlings were sampled at fifth and fifteenth day. The plants were grown in a climate chamber (Conviron), under the following conditions: 21/18 °C day/night; 125 µE/m^2/s, 75% RH and light period 12 h/day and 12 h/night.

2.2 RNA isolation

For RNA-Seq analysis, five plants of uniform appearance were collected at each analyzed stage, ground to a fine powder in liquid nitrogen, and stored at -80 ºC until further use. Three biological replicates were conducted. In each case, total RNA was isolated with the RNeasy Plant mini kit (Qiagen, UK) according to the manufacturer’s instructions. RNA quality and concentration were determined using NanoDrop ND- 100 (NanoDrop technologies) and by Agilent 2100 Bioanalyzer. We discarded any samples with an RNA integrity number (Schroeder *et al.,* 2006) lower than 7.0 or an rRNA 28S/18S ratio above 0.7.

2.3 cDNA library preparation

Double-stranded cDNA was synthesized with Takara’s PrimeScript RT Reagent Kit (Clontech, USA) using random hexamer primers and 10 µg of mRNA enriched in short fragments (200 bp). After end repair with T4 DNA polymerase and Klenow DNA polymerase (60 min at 16°C), Illumina adapters were ligated, and the fragment population was enriched by PCR amplification. One library was constructed per biological replicate and stage analysed.

Sequencing was conducted using an Illumina High-Seq 2000 platform using paired-end 50 bp (long reads) in fastq format.

2.4 Preprocessing of Illumina reads

The RNA-seq reads were initially pre-processed includes filtering of low-quality sequences, identification of specific features (such as poly-A or poly-T tails, terminal transferase tails, and adaptors), removal of contaminant sequences (from vector to any other artefacts) and trimming the undesired segments using a Seqtrim pipeline (http://www.scbi. uma.es/seqtrimnext; (Falgueras *et al*., 2010) available at the Plataforma Andaluza de Bioinformatica (University of Malaga, Spain). We use the Bowtie2 (Langmead and Salzberg, 2012) tool to take a collection of short reads and search for each read's best alignment to a reference genome and transcriptome of *P. trichocarpa* (http://www.phytozome.net).

2.5 Differentially expressed genes (DEGs)

Digital quantification of gene expression was performed with TMM (Trimmed mean of M-values) normalization method (Robinson and Oshlack, 2010). Only uniquely mapped reads were used to estimate expression levels. Screening of differentially expressed genes in pairwise comparisons was performed using four algorithms (DESeq2 (Love *et al.,* 2014), limma (Smyth, 2005), edgeR (Robinson *et al.,* 2010) and NOISeq (Tarazona *et al*., 2011) based on DEgene HUNTER by (González- Gayte *et al*., 2015). Afterwards, p-values were corrected for multiple testing using the False Discovery Rate (FDR) method (Benjamini and Hochberg, 1995). We defined differential expression as an absolute fold change cutoff value of 1 in $\log_2$ scale and a FDR cut-off ≤ 0.05. The resulting gene counts table was subjected to differential expression analysis for the contrasts control versus C1_T1, control versus C1_T2, control versus C2_T1, control versus C2_T2 using the Bioconductor packages DESeq2, edgeR, NOISeq and limma. In order to combine the statistical significance from multiple algorithms and perform DEgen Hunter-analysis.

2.6 Time course

This analysis was performed on normalized gene data and grouped based on their behavioral profile with the maSIGpro program (Microarray Significan Profile) (Conesa *et al*., 2006), which uses a linear regression analysis to model gene expression. A sequential analysis was performed for all treatments (1: low concentration and 2: high concentration) at early and late time (5 and 15 days). We thus obtained a classification of the genes according to 4 expression patterns. Not all genes are included in the four groups, as this program ranks the most representative genes in each case.

2.7 Analysis of gene co-expression networks

These analyzes were performed to detect potential candidates for participation in PCB degradation pathways. The exNet tool (http://popgenie.org/exnet) was used to visualize the detected co-expression networks. The correlation measures are given by a CLR (Context Likelihood of Relatedness), based in turn on a mutual information value (MI), that is, a probability of relationship between two genes (Shiquan Wu and Jing Li, 2007). The exNet program (http://popgenie.org/exnet) generates an image where the nodes that are shown as

Vol. 09, No. 03; 2024

ISSN: 2456-8643

yellow squares are the transcriptional factors and the green circles represent the genes. Cytoscape 3.2.1 was used as a platform for the visualization of co-expression images (Shannon *et al*., 2003).

2.8 Validation of gene expression data

To validate the RNA-seq data, transcripts from thirteen genes were quantified by real-time PCR analysis. Primers were designed using Oligo Analyzer 1.0.3. The genes were selected because it's showed high expression levels and for its candidature. One ng of cDNA was utilized in triplicate for 40 cycle, two step PCR in an (ABI PRISM® 7000/7700/79OOHT_7300 Applied Biosystem) using the following program: 95°C for 2 min, 40 cycles of 95°C for 10 s, 59°C for 10 s and 72°C for 10 s, using SYBR Green Master Mix (Applied Biosystems) and 200 nM of each primer 0.4 µl of each primer, 1 µl of first-strand cDNAs and 10 µl of SYBR Green and 8.3 µl of H_2O. Triplicate Q-RT-PCR runs were performed for each target gene. Amplicon size (64- 255 pbs) and reaction specificity were confirmed by product dissociation curves. 18S rRNA expression was measured as internal control. Approximately 13 genes were selected from among up regulated genes PCR amplification was performed with gene-specific primers selected and 18S as control (Supplemental Information).

3. RESULTS AND DISCUSSION

3.1 Growth of *Populus in vitro* on PCB (Aroclor 1221) media

Populus exhibit many stress characteristics, such as change of seedling color and reduction of root growth when they grew on tubes and medium containing low, medium and high concentration of Aroclor 1221 (Figure 1). Compared with the control, Aroclor concentration of 200 mg/L continued with the root elongation and biomass. As seen in Figure 1C, at 200 mg / L at 15 days the root length growth was 25% with respect to the control. The same pattern is observed in Figure 1B in the evaluation of dry leaf weight (biomass) where the amount of biomass was lower due to the decrease in growth considering a 30% difference of the treatments against the control at 15 days. These two concentrations and times were considered for subsequent transcriptomic analyzes quality and quantity results of RNA samples were tested with Bioanalizer in order to give adequate RNA quality for RNA-seq.

In vitro plants were tested under controlled conditions of light and temperature. The *in vitro* culture was chosen to ensure that microbial interference would not occur in the results. The tests consisted in the evaluation of leaf and root growth of hybrid poplars in the presence of different concentrations of Aroclor 1221 diluted in sterile DMSO from 0 (control) to 200 mg / L and at two times, 5 and 15 days. It was difficult to establish the optimum conditions of treatment a priori, since the tolerance varies greatly according to the species and the physiological state of each specimen, not to mention the conditions of the environment (Yang *et al*., 2013). Comparisons between treated plants and control plants revealed significant differences in some quantitative variable. Plant growth in general and root system decreased as PCB concentration and time of exposure increased. Symptoms of chlorosis and foliar necrosis also appeared. These effects agree with typical responses of cell stress (Yang *et al*., 2013). In our case, the growth and morphological analyzes have been used to evaluate the poplar response to different levels of PCBs and to select the optimal experimental conditions for the treatments.

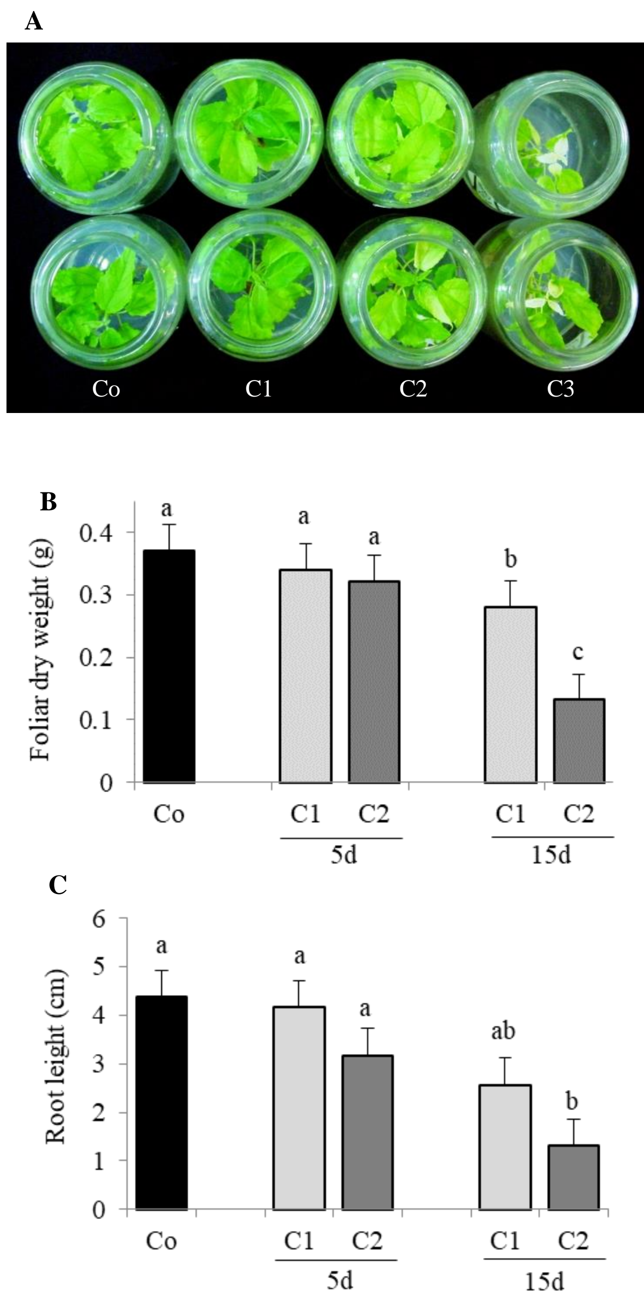

Figure 1. Effect of Aroclor 1221 treatment on *Populus* seedling growth. A) Seedling growth was sowed on 50, 100 and 200 mg/l at 5 and 15 days. B) Foliar dry weight. C) Root growth. Foliar Dry weight and root length was measured after 15 day growth. Data points show mean standard deviation, n=20 for every concentration. C1= 50 mg/L, C2= 100 mg/L, C3= 200 mg/L

3.2 Quality and robustness of the sequencing

In model species such as Populus thrichocarpa readings obtained by sequencing the RNA are mapped against its own transcriptome. Generated cDNA sequencing 11,950,000 minimum and maximum readings of 12, 6109,271, which corresponds to an average of 585,570,000 nucleotide per sample. In all cases, more than 60% of the readings could be mapped a 61.85% minimum and a maximum of 78.78%. Also, the quality of the sequencing was very good, given that over 98% of the raw readings can be considered clean readings. An important step before the analysis was data pre-processing to obtain the clean reads. Removing artefacts from RNA-seq datasets improves the read quality, which, in turn, improves the accuracy and computational efficiency of the assembly (Martin and Wang, 2011).

Pre-processing of the initial sequencing readings was performed to increase its quality and eliminate artifactual elements (Falgueras *et al*., 2010). There are several bioinformatic tools for this purpose, such as Trim-Seq, TrimEST, VectorStrip, Lucy and SeqClean (Scheetz *et al*., 2003; White *et al*., 2008; Chou and Holmes, 2001; Li and Chou, 2004). Compared to these programs, the one used here (SeqtrimNEXT) is able to maintain high performance information analysis, even with short sequences, being flexible in input and output formats. It is also accessible by any user through a web interface and provides more accurate results than other programs (Falgueras *et al*., 2010). In our case SeqtrimNEXT turned out to be very efficient for the elimination of low quality readings, adapters, very complex readings, Poly A/T, contaminated sequences etc. The average percentage of readings was 13%, while the percentage of readings discarded by the algorithm of BGI was only 3%. This is because as we mentioned above, SeqtrimNEXT is a more complex and strict program.We detected, 31,037 in treatment 50 mg/L of Aroclor 1221 at 15 days (low concentration and long time), and 30,582 in treatment 200 mg / L at 15 days (high concentration and long time); 28,792 in treatment 50 mg/L of Aroclor 1221 at 5 d (low concentration and low time), 31,122 in treatment 200 mg/L de Aroclor a 5 d (high concentration and low time), and 31,418 transcripts in the Control.

3.3 Quantification of gene expression

By performing pair-wise comparisons, we could identify the differences in transcript abundance between all the conditions analysed. The filtering process generated a good amount of expressed and unpressed or repressed genes. To evaluate the overall effect of the contaminant were compared each with respect to the control samples. The levels of differential expression between the different conditions analyzed were evaluated. Comparisons showed 87 overexpressed and 517 repressed genes (Control vs. 50 mg / L to 5 d); 737 over-expressed and 1001 repressed (Control vs. 200 mg / L to 5 d); 1319 over-expressed and 1683 repressed (Control vs. 50 mg / L at 15 d), 3366 over-expressed and 3352 repressed (Control vs. 200 mg / L at 15 d). As a result, the numbers of differentially expressed transcripts are increasing as the concentration and exposure time.

The differential expression analyzes performed were based on reading count statistics mapping on the transcriptome and the genome of *P. trichocarpa*. The poplars used in our study are hybrids *P. tremula x P. alba*, of the same genus and therefore closely related. That is why the mapping radius is smaller than if the species used had been *P. trichocarpa*. Transcripts having a relative abundance greater than 2 relative to the control were selected for the subsequent analyzes. The process generated a good amount of expressed and unpressed or repressed genes.

Vol. 09, No. 03; 2024

ISSN: 2456-8643

It is clearly seen that the number of differentially expressed genes increases as concentration and time also increase. This is probably due to the fact that there is increased cell stress and more genes are activated. To achieve our goal of finding candidate genes, we decided to analyze only the over-expressed SDSs. In addition, we have focused on the genes that are interconnected in the four treatments to have greater reliability.

3.4 Genes that interact in all four treatments

After obtaining the differentially expressed genes, several gene lists were obtained. Thus, a good way to select those genes that participate when an experimental process has been triggered is by paying attention to those genes involved in the response to the pollutants according to the algorithm used. In the comparison Control vs. 50 mg / L at 5 d interact 66 genes, in the Control vs. 200 mg / L at 5 d interact 137 genes, in the comparison Control vs. 50 mg / L at 15 d interact 403 genes and in the Control vs. 200 mg / L at 15 d interact 1,106 genes (Figure 2).

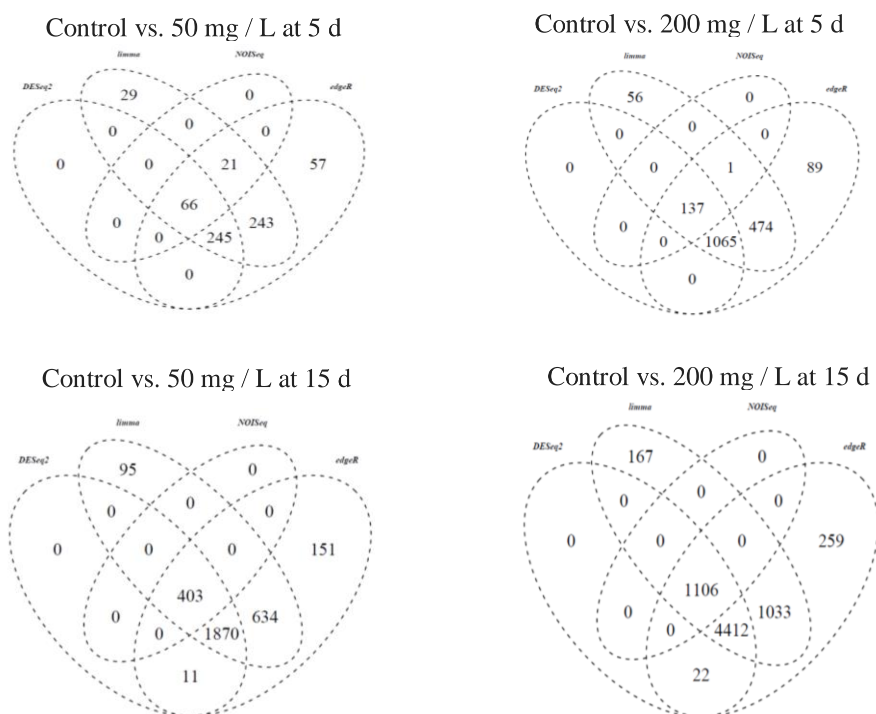

Figure 2. Venn diagram of all comparisons. In the center the interaction of the DEGs that are the same in the four DEGen Hunter programs (limma, DESeq2, NOISeq and edgeR) is shown, Control vs. 50 mg / L at 5 d has 66 interactions. Control vs. 200 mg / L at 5 d 137 interactions, Control vs. 50 mg / L at 15 d 403 interactions and Control vs. 200 mg / L at 15 d 1,106 interactions.

We generate in particular 4 groups or clusters, which show 4 different behaviors, but the groups with the highest number of genes expressed positively in group 3 and 4. In group 3, 2,229 are grouped whose expression increases over time. In this group are thus found the most expressed genes in both times and treatments. In group 4, 2,054 genes are included whose

expression initially increases with respect to the control but then decreases. In the case of treatment 2 they go from 85 to 75, and in in treatment 1 from 55 to 50.

3.6 Genes related to the metabolism of PCB degradation

Table 1 shows the candidate genes to participate in the response to Aroclor in *Populus* plants. Only expressions that presented a difference of 2 times with the reference were considered. (Absolute Value of Relation between the log2 values of the samples to compare were greater than 1).

Table 1. Genes with totential contribution in the degradation metabolism pathways of Aroclor 1221. Three phases of detoxification according to RNA-seq data (Log2 ratio) are proposed.

Gen name	Locus	Arabidopsis ortholog	log2 (200_15d/Co)	FDR
Phase I (Transformation: Oxidation, hydrolysis and reduction reactions)				
Polyphenol oxidase	Potri.001G388900.1	--	2,45	2,52E-295
Glyoxal oxidase	Potri.005G235500.1	At3G57620	4,62	1,07E-05
Thioredoxin	Potri.001G416500.1	--	3,62	1,33E-14
	Potri.010G059400.2	--	4,15	0,00070933
Aminocyclopropane-1-carboxylate	Potri.002G163700.1	At3G61510	5,98	1,61E-16
Cinnamyl alcohol dehydrogenase	Potri.009G063100.1	At3G03980	2,41	5,00E-28
Short-chain deshidrogenase	Potri.013G059100.2	At3g03980	1,51	1,36E-05
D-Lactate dehydrogenase E	Potri.001G462200.1	At1g30700	4,19	2,92E-32
Inosine-5-Monophosphate dehydrogenase	Potri.009G099200.1	--	1,65	0,00031137
Xanthoxin dehydrogenase	Potri.004G199900.1	--	4,37	6,49E-138
Naringenin/Flavanone 3-dioxygenase	Potri.011G150100.1	--	2,94	2,43E-06
2,4-dihydroxy-1,4-benzoxazin-3-1-glucoside Dioxygenase	Potri.010G107500.1	--	1,26	3,32E-91
Flavin monooxygenase	Potri.002G254200.1	At4g28720	2,08	8,10E-05

4,5-DOPA dioxygenase extradiol (ring opening)	Potri.004G135300.1	--	2,06	4,52E-228
Cytochrome P450 monooxygenase (CYP82G1)	Potri.004G106600.1	At3G25180	2,78	2,73E-57
	Potri.003G173500.1	--	3,08	2,68E-07
	Potri.008G099100.1	--	4,24	2,90E-196
Ubiquitin ligase E3 Zinc finger (C3HC4)	Potri.011G063100.1	--	2,48	0,00010991
	Potri.004G042700.1	--	2,62	2,98E-22
Nitrate reductase/NADH-cytochrome B5 reductase	Potri.002G088600.1	At1g77760	2,93	0
Glutathione reductase	Potri.003G178200.1	At3g24170	1,34	3,67E-16
Isocitrate lyase	Potri.007G122900.1	At3g21720	11,41	8,42E-49
AMP-activated protein kinase	Potri.011G122000.1	At4g27460	4,22	3,04E-08
Chitinase	Potri.012G033900.1	--	3,49	1,49E-119
	Potri.006G188400.1	--	4,51	3,34E-176
Zeta-carotene desaturase	Potri.005G177700.1	--	2,03	2,46E-27
	Potri.003G213700.1	--	3,62	4,92E-232
Cold Shock Protein	Potri.004G172600.1	At4G38680	1,28	0,000121511
Phase II (Conjugation) Deactivation by the formation of covalent bonds with endogenous hydrophilic molecules such as glucose, malonate, glutathione, or carboxylic acids (glucosyl/glutathione transferase)				
Glucosyl transferase	Potri.010G075400.1	At2g38870	10,25	0,00053911
UDP-glucose:(indole-3-yl)acetate beta-D-Glucosyltransferase	Potri.002G236500.1	--	2,30	1,52E-42
	Potri.014G146000.1	--	3,27	8,51E-298
UDP-glucosyl transferase subfamily 73C	Potri.001G303000.1	--	2,19	1,30E-08
Homocysteine S-	Potri.008G1	At3G22	3,68	1,53E-

methyltransferase	55900.1	740		20
Omega-hydroxypalmitate O-feruloyl transferase	Potri.008G180400.1	--	2,15	6,71E-60
	Potri.010G186300.1	--	2,08	8,09E-05
UDP-glucotrasferase	Potri.006G055600.1	--	4,54	2,87E-241
Glutathione transferase (GST25)	Potri.012G050100.1	--	9,39	0,00015295
	Potri.010G060900.2	--	3,02	3,00E-12
Xyloglucan:xyloglucosyl transferase	Potri.018G095200.1	--	3,22	1,00E-06
	Potri.005G007200.1	--	4,68	5,84E-06
2-amino-2-carboxyethyl transferase	Potri.005G048400.1	--	2,11	5,16E-05
1 aminocyclopropane carboxylate	Potri.002G163700.1	At3G61510	5,97	3,07E-14
Fase III (Compartimentación) Exportación de los conjugados , ya sea a la vacuola al apoplasto (a través de transportadores)				
Chlorine Channel 7	Potri.018G124100.1	At5g33280	1,04	0,00078673
MFS Conveyor, OPA Family	Potri.018G115000.1	At2g13100	1,14	2,92E-05
MFS Conveyor, SP Family	Potri.010G089800.1	--	1,82	1,49E-107
MDR (Multidrug Resistance) Conveyor ABC Family	Potri.012G033400.1	--	1,45	0,00033333
	Potri.008G003300.1	At3g54540	1,09	2,76E-28
Bile acid:Na+ symporter, BASS Family	Potri.003G054500.1	--	8,25	0,00053958
Factores de Transcripción				
Zinc finger transcription factor of the C2H2 type	Potri.008G051200.1	At2g28710	3,21	2,90E-08
	Potri.010G209400.1	--	3,76	8,41E-28
Homeobox-leucine zipper transcription factor	Potri.012G023700.1	--	2,04	1,23E-50
	Potri.014G103000.1	At2g46680	3,24	7,08E-43

NAC transcription factor	Potri.001G404100.1	At3g15500	2,30	4,26E-58
	Potri.013G054200.1	--	3,56	6,46E-180
TGA transcription factor, STIP1.	Potri.006G107600.1	At5g01830	2,79	5,18E-40
Bzip transcription factor	Potri.009G164300.1	At1G08320	2,01	0.0115341
Zinc Finger , PMZ, SAP12	Potri.011G138500.1	At3g28210	1,58	1,83E+00
WRKY transcription factor 75	Potri.015G099200.1	At5G13080	1,54	0.00365706
	Potri.003G169100.1	At5G13080	1,20	4,83E-04
	Potri.T043800.1	At5G13080	2,55	8,47E-10
Basic helix-loop-helix BHLH071	Potri.001G141100.1	At5G46690	2,17	0.0285048
GRAS2, SCL14 transcription factor	Potri.009G032800.1	At1G07530	1,32	0.00838648
MYB3 transcription factor	Potri.013G109300.1	At1G22640	1,50	3,64E-119
MYB Proto oncogen transcription factor	Potri.019G081500.1	AT1G22640	1,38	1,41E-10
	Potri.008G122100.1	--	3,66	2,41E-58

ISSN: 2456-8643

3.7 Validation with qRT-PCR

For the validation of the data of RNA seq 15 genes were selected, having as criterion to choose the most over-expressed and that would be considered possible candidates of mechanisms of degradation. The differential expression at 15 days at a concentration of 200 mg/L was checked by a validation using qRT-PCR. The final results are reported as fold change. For this validation we designed previously specific oligos for each gene under study. In addition, the validation of 15 genes by qRT-PCR and RNA-seq correlation results strongly support the feasibility of our study and support its applicability to hybrid poplar genotypes. As part of the differential expression analysis it was necessary to generate a matrix of four elements for all comparisons (control vs treatments). In order to increase the specificity and reliability of our analysis, we also consider the differentially expressed genes that interact in the four treatments, according to four different statistical algorithms (González-Gayte *et al*., 2015).

3.8 Gene expression coexpression analysis

From the 90 genes in Table 1 a genetic co-expression analysis was performed. The analysis revealed 162 co-expressions between genes and transcription factors (Figure 3)

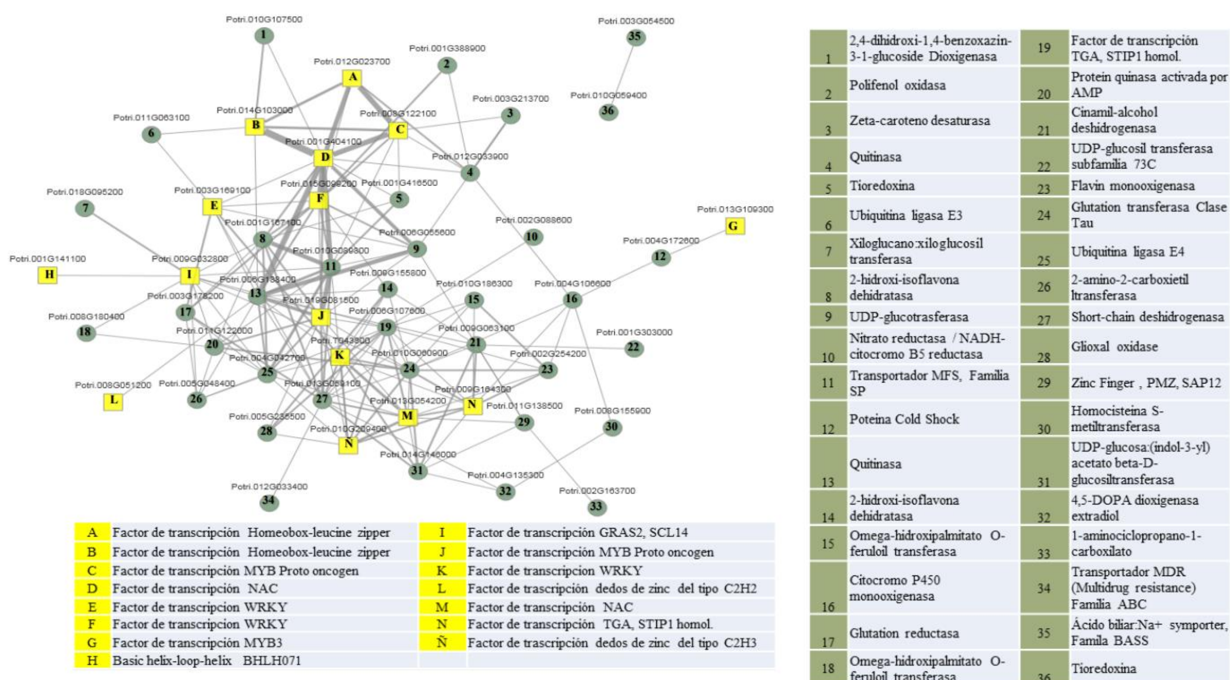

Figure 3. Co-expression network of the DEGs involved in the presumed PCB stress process. Candidate genes are marked in red next to the gene acronym. AMP (protein kinase), GSR (Glutathione reductase), CHIA (Chitinase), LETM (leucine zipper EF hand), MFS (MFS Transporter), UDP_GT (UDP glucotransferase), TRX (Thioredoxin).

3.9 Selection of genes and transcription factors

ISSN: 2456-8643

The analysis of gene interaction in the four treatments was done by four statistical packages (edgeR, limma, NOISeq and DESeq2) in order to have a greater validity. The genes that are in the interaction are, in their majority, genes with function of oxidation-reduction, hydrolysis, conjugation, transport, compartmentalization and transcription factors whose participation will be analyzed later, being graphically useful the use of Venn Diagrams and Heat maps. This strategy has also been used by Ariani *et al*., (2015) who have used the genes that are grouped at the intersection. However, they only used a statistical package (DESeq) so our study is statistically more robust.

On the other hand, to understand the transcriptional changes over time and the genes that are grouped before a given event (contamination with PCBs) a sequential analysis was performed. The results obtained have helped us to better understand the decontamination mechanism. This strategy was proposed by Nueda *et al*., (2009) and has been applied by several authors (Prado-López *et al*., 2010; Rizza *et al*., 2012). We identified four groups or profiles of gene expression, which indicate that the grouping of sequential analysis that gives us greater and better information was Group 3 due to a behavior that goes up according to the concentration of pollutant and time increase the amount of genes Over-expressed. In this group more than 18 ABC transporters, 10 MDR transporters, 5 laccases, 4 catalases, 4 thioredoxins, 7 glutathione reductases and more than 26 cytochromes P450 have been found. This suggests that these processes are well represented, especially in the longest treatment (15 days). In addition, sequential analysis indicates that in the short time (5 days) only signaling genes, kinase receptors and factors that could participate in detection and primary signaling from detoxification of PCBs are activated. Certainly, the genes in the longer treatment may prove to be good candidates classified in Stage I: Transformation (as P450), Stage II: Conjugation (GST and UGT), and Stage III: Compartmentalization Conveyors (ABC, MDR etc.). Finally, the expressed and normalized genes were subjected to co-expression analysis. Being integrated a number of genes can provide models of transcriptional networks that control the gene expression of a particular mechanism, in this case the mechanism of metabolism of PCBs.

3.10 Genes and Transcription Factors Involved in PCB Metabolism

Gene expression related to the metabolism of PCBs in poplar and its regulation is coordinated by 35 genes and 15 transcription factors which are key genes or possible transcriptional keys with phytoremediation potential. It also allows us to observe a chain of genes linked by their co-expression in the presence of PCBs. In Figure 3, a marked gene co-expression of *Glutathione transferase* is observed, this is because the synthesis of glutathione is regulated by the family of enzymes known as *Glutathione-S transferases* (GSTs). It is proven that in the presence of some contaminants, a complex is formed with the GST molecule, which interacts with enzymes that facilitate its degradation, such as *Glutaminyl transpeptidase* (Brazier *et al*., 2005). In the co-expression analysis a GST gene (GSTU25) (Potri.010G60900) was found which is very likely to be related to the detoxification of PCBs.

A recent study mentions that tripeptide glutathione is the most important antioxidant in plants which is related to species detoxification (ROS) and REDOX signaling (Zechmann, 2014). Other studies suggest the relationship of oxidative stress (REDOX mechanisms) with the presence of xenobiotics (Mylona *et al*., 2007).

Vol. 09, No. 03; 2024

ISSN: 2456-8643

Consequently the induction and coordination of both responses (detoxification and antioxidant) are of great importance for efficient defense. Detoxification of xenobiotics typically shows activation through hydrolysis or oxidation catalyzed by the cytochrome P450 monooxygenase, followed by a covalent attachment of endogenous hydrophilic molecules such as glutathione, glucose or malonate to form more soluble compounds and less toxic conjugates (Zechmann 2014). In our results the induction of Cyt P450 (Potri.004G106600) is remarkable. The P450 system comprises a total of 90 genes that are encoded in the genome of *P. trichocarpa*. The encoded proteins form a superfamily whose members contain the heme cofactor that catalyzes the oxidation of organic molecules (Olsen *et al*., 2015). In addition to hemoproteins the co-expression system identified flavin monooxygenase (Potri.002G254200), UDP-glucosyltransferase (Potri.001G303000) and cinnamyl alcohol dehydrogenase (Potri009G063100), among other genes. The *Flavin-monooxygenases* occupy the second place in importance in the metabolic oxidations. These enzymes are involved in the oxygenation of nitrogenous compounds (with the formation of N-oxides), organophosphates and organosulfurites. Unlike cytochromes, they are *flavin-adenosine dinucleotide* (FAD) enzymes as a cofactor to reduce one of the two oxygen atoms. Its catalytic mechanism of action is unique: unlike cytochromes P450, the interaction of the xenobiotic with the enzyme is much looser. The oxidation occurs through an oxidizing reagent intermediate generated at the same catalytic center of the enzyme (*hydroperoxy-flavin monooxygenase*).
In that same line there is a key enzyme called *Short Chain dehydrogenase* (Potri.013G059100), which is coexpressed with *Glutathione transferase* (Potri.010G60900) and an MDR transport protein (Potri.012G033400) which is by homology MRP14. The latter relates to transport mechanisms for the exclusion of xenobiotics and their metabolites (Fekete *et al*., 2015). Such transport is an integral part of the detoxification mechanism. Plants do not have efficient systems to excrete compounds in the medium, so they have to store potentially toxic compounds in vacuoles. In addition, there is a great deal of research that reveals that MDR is not only involved in detoxification, but also in cellular regulation of storage (Gaedeke *et al.,* 2001); AT3G59140 (AtMRP14; MRP14) has been found to be induced more than 2.5 fold (fold change) in response to Benzoxazolinone in *Arabidopsis* (Baerson *et al.,* 2005). The above mentioned confirms that the MDR transporter Potri.012G033400 (34) is related to the contamination process since it has orthologous to AT3G59140 (MRP14).

SDR proteins constitute a dependent superfamily of NADH/NADPH-dependent dehydrogenases. These enzymes have a low sequence homology, but a good conservation of the 3D structure, suggesting similarities also in the catalytic mechanisms. The high variability of the active center explains the wide range of substrates that SDRs can process. The main functional characteristics of these proteins include the ability to interconvert carboxyl and hydroxyl groups and the reduction of C-and N-N-double bonds (Jörnvall *et al*., 1995). Another interesting feature of SDRs is their high similarity in structure and possibly in function with bacterial dehalogenases of halohydrides. Deshalogenases catalyze the nucleophilic removal of a halogen, substituted by a hydroxyl (Van Hylckama Vlieg *et al*., 2001). These enzymes are very interesting for the bioremediation of aliphatic halides in the soil. Their genes are receiving increasing attention from the biotechnological point of view (Kavangh *et al.,* 2008). To date no mechanism of dehalogenation has been described in plants.

Figure 3 shows that the coefficient of co-expression is high, and it is possible to visualize the width of the binding lines. The MFS transporter (11) (Potri.010G089800) belongs to one of the five families of MDR transporters which maintains co-expression networks with four TRX (5) (Potri.001G416500), leucine zipper EF hand (8) Potri.001G187100 and Kinasa (13) (Potri.006G138400), thus showing its central role in the process. This suggests that in addition to the ABC family, the major superfamily of facilitating transporters (MFS) also prevents accumulation of toxic compounds (Pao *et al.,* 1998; Reedy *et al.,* 2012).

Previously it was considered that the MFS transporters were only concerned with the secretion of endogenous toxins (Reedy *et al.,* 2012). However, there are studies that have demonstrated that MFS transporters of *Candida albicans* and *S. cerevisiae* are also involved in protection against exogenous compounds, such as sterol demethylation inhibitors (Calabrese *et al.,* 2000; Hayashi *et al.,* 2002). Other studies in *Botrytis cinerea* revealed the existence of an MFS transporter, called Bcmfs1. The main function of this protein is to metabolize xenobiotics. They are flavin NADPH-dependent class A monooxygenases, involved in the desulfurization pathway of organic compounds, coupling NADH oxidation with substrate oxidation, by reducing FMN to FMNH2. They catalyze oxygenation reactions from molecular oxygen, which acts as a substrate (Hayashi *et al.,* 2002). In this same network Figure 3 is a protein kinase AMP (20) (Potri.011G122000). These proteins are activated by metabolic stress and xenobiotics, which interfere with the generation of catabolic ATP (Hardie, 2007).

The response to xenobiotics depends on a single transcription factor (Ekman *et al.,* 2005; Ramel *et al.,* 2007; Wang *et al.,* 2012). We have found that MYBs and WRKYs are involved in the regulation of such response (Singh *et al.,* 2002). Ramel *et al.,* (2012) point to At5G13080 (WRKY75) as a transcription factor associated with stress tolerance by xenobiotics. This transcription factor is homologous to Potri.003G169100 (E), Potri.T04800 (K), and Potri.015G099200 (F) confirming the participation of these factors in response to PCBs. The induction of WRKY75 encodes genes that are induced by ROS effectors such as the methylated viologen dichlorate (paraquat), the toxin from the fungus *Alternaria alternata* (tenuazonic acid), and 3-amino-triazole (amitrol) (Gadjev *et al.,* 2006). Ochratoxin A induces WRKY75 (Wang *et al.,* 2012), which is associated with atrazine defense response. Several cis-regulatory elements have been identified in the promoter regions of atrazine-responsive genes (Ekman et al. 2005; Ramel *et al.,* 2007; Wang *et al.,* 2012). It has been found that, binding sites to the TAACTAAC consensus sequence of MYB3 have been related in response to stress (Bang *et al.,* 2008). In our co-expression analysis a transcription factor MYB3 Potri.003G109300 (G) was found indicating its participation in the response to PCBs. It is interesting to co-express it with the gene PtCSP4 (Potri.004G172600) (12), a gene never previously related to xenobiotic responses.

On the other hand, Baerson *et al.,* (2005) and Fode *et al.,* (2008) have already emphasized the central role of TGA factors in the response to xenobiotics. The transcription factor TGA-type STP1 (Potri.009G164300) (N) co-expression analysis could participate in the degradation pathway of xenobiotics. In addition, a GRAS (SCL14) transcription factor Potri.009G032800 (I) was found in our analysis. SCARECROW-like 14 (SCL14) is a member of the GRAS family that responds to herbicides (Behringer *et al.,* 2011).

Helix-loop-helix (bHLH) transcription factors regulate ROS distribution (Tsukagoshi *et al.,* 2010). These genes in animals are nuclear receptors with transcriptional activity or are part of transcription factor complexes (Baker, 2005), which are closely linked to environmental signals.

Certainly, AhRs (aryl hydrocarbon) receptors recognize organic pollutants. The AhRs are members of the bHLH-Per-ARNT-Sim family of transcription factors (bHLH-PAS) (McMillan and Bradfield, 2007). In our co-expression analysis we found a bHLH07 (Potri.001G141100) (H). This finding confirms the participation of bHLH07 in the regulation of the response to xenobiotics. AhR is a transcription factor activated by ligand binding, through its heterodimerization with a second protein called AhR nuclear translocator (ARNT, Aryl hydrocarbon Receptor). The formation of this transcriptionally active heterodimer induces the expression of target genes encoding enzymes involved in detoxification of both phase I (cytochrome P450, CYP450) and phase II (transferases). The binding of AhR to its ligands leads to receptor activation, which leads to changes in cellular compartmentalization. In the absence of ligand, the latent form of AhR is associated with two molecules HSP90 (Heat Shock Protein 90), a molecule of chaperonin p2 and protein XAP2 (Hepatitis B virus X-Associated Protein 2), also known as ARA9 (AhR-Associated protein 9) or AIP (AhR-Interacting Protein) (Bessede et al. 2014). Upon binding of the ligand, AhR rapidly accumulates in the cell nucleus where it forms a transcriptionally active heterodimer with TNA (AhR Nuclear Translocator). This heterodimerization dissociates the HSP90-XAP2-p23 complex from AhR. The active AhR / ARNT heterodimer binds to regulatory elements called XREs or DREs (Xenobiotics or Dioxin Response Elements) located in target gene enhancer / promoter regions, thus enhancing transcription thereof (Petersen *et al.,* 2003). The established consensus sequence for XRE is 5 'GCGTG 3' (Huang and Elferink, 2012). By means of the database analysis we have been able to identify this consensus sequence in the gene PtCyp_P450 (Potri.004G106600), PtSDR (Potri.013G059100), 4,5-DOPA dioxygenase extradiol (Potri.004G135300) and PtXGT transferase (Potri.005G007200) (Figure 4) potentially regulated by an AhR type factor. A scheme of the proposed mechanism of action for the transcriptional activation of AhR-dependent genes is shown in Figure 4. The target genes identified encode enzymes involved in metabolic detoxification of both Phase I (P450, flavin monooxygenases, cinnamyl alcohol dehydrogenases, etc.) and Phase II (UDP-glucosyltransferases, glutathione-S transferases, etc.) and Phase III compartmentalization.

Vol. 09, No. 03; 2024

ISSN: 2456-8643

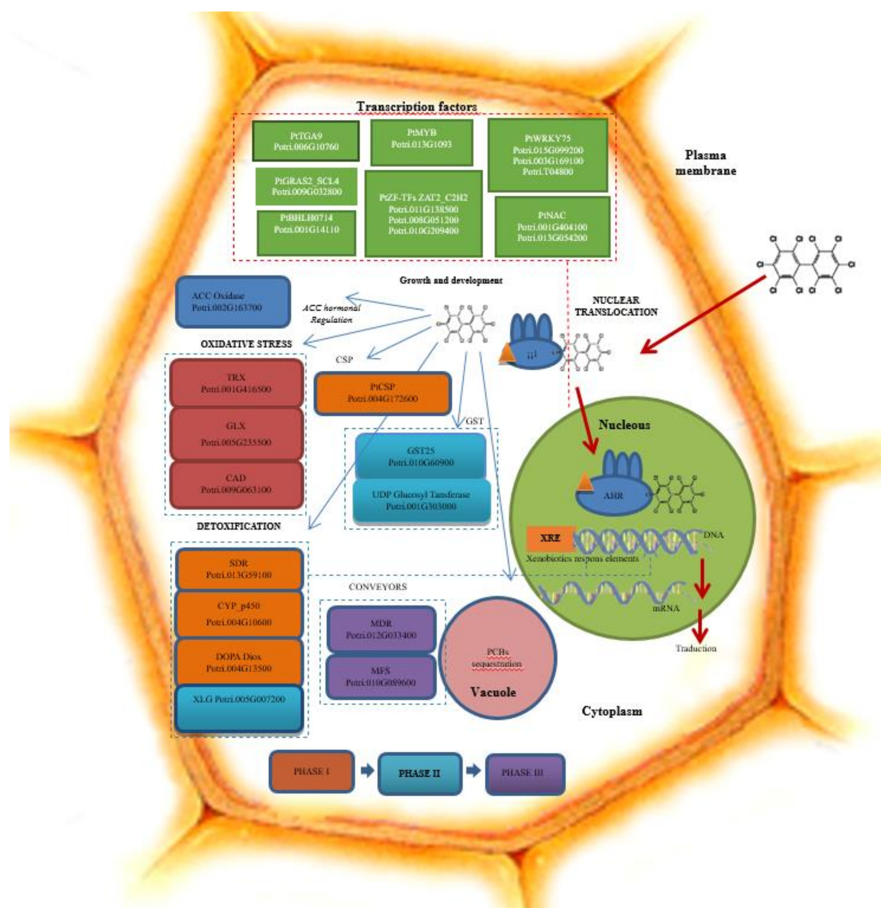

Figure 4. Hypothetical model of the regulatory network of genes and transcription factors that respond to PCBs in Populus. Signal transduction mediated by the AhR/ARNT heterodimer showing the changes in the cellular distribution of the components after activation by ligand binding.

4. CONCLUSIONS

It is the first study to describe a transcriptomic analysis in Populus in order to elucidate the mechanism of action of one of the most dangerous organic pollutants in the world, PCBs. The transcriptomic results add to current knowledge about the stress response in Populus. Furthermore, the data generated can be used as a reference transcriptome for subsequent studies in Populus or other forest species to address the molecular mechanisms underlying contaminant susceptibility and stress resistance. Genes have been identified that presume an important participation in the metabolism of PCBs. Which will be useful in many aspects of plant and environmental biotechnology. These genes may give rise to new biomarkers that are related to gene networks that respond to PCB stress. In particular a Cytochrome P450 (Potri.004G106600), GST (Potri.010G060900), SDR (Potri.013G059100), 4,5- DOPA extradiol dioxygenase (Potri.004G135300), XGT transferase (Potri.005G007200), 1-amino cycle propane carboxylate (Potri.002G163700), MDR Carrier (Potri.012G033400), MFS Carrier (Potri.010G089800) and

CSP4 (Potri.004G172600). Transcription factors that participate in the metabolism of PCBs have been identified and a metabolic pathway is proposed. Belonging to WRKY75 (Potri.015G099200, Potri.003G169100 and Potri.T043800), MYB3 (Potri.013G109300), GRAS2 (SCL14) (Potri.009G032800), ZF-TF (Potri.008G051200), bHLH071 Potri.001 G141100, NAC Potri .001G404100 and Potri.013G054200, TGA Potri.009G164300, Zinc Finger C2H2 Potri.008G051200 and Potri.010G209400, Zinc Finger ZAT12 Potri.011G138500.

REFERENCES

Abraham WR., Nogales B, Golyshin PN, Pieper DH, Timmis KN (2002) Polychlorinated-biphenol-degrading microbial communities in soils and sediments. Curr Opin Microbiol 5, (3):246-253. doi: 10.1016/S1369-5274(02)00323-5

Anyasi RO and Atagana HI (2011) Biological remediation of polychlorinated biphenyls (PCB) in the environment by plants and microorganisms. Afr J Biotechnol 10, 18916-18938. doi: 10.5897/AJB10.557

Ariani A, Di Baccio D, Romeo S, Lombardi L, Andreucci A, Lux A, Stephen D, Luca H (2015) RNA Sequencing of *Populus x canadensis* roots identifies key molecular mechanisms underlying physiological adaption to excess zinc. PLoS ONE 10(2): 1-20. doi: 10.1371/journal.pone.0117571

Ashburner M, Ball CA, et al. (2000) Gene ontology: tool for the unification of biology. Nat Genet 25(1):25-29. doi: 10.1038/75556

Baerson SR, Sanchez-Moreiras A, Pedrol-Bonjoch N, Schulz M, Kagan IA, Agarwal AK, Reigosa MJ, Duke SO (2005) Detoxification and transcriptome response in *Arabidopsis* seedlings exposed to the allelochemical benzoxazolin-2(3H)-one. J. Biol Chem 280: 21867-21881. doi: 10.1074/jbc.M500694200

Baker ME (2005) Xenobiotics and the evolution of multicellular animals: emergence and diversification of ligand-activated transcription factors. Integr Comp Biol 45:172-178. doi: 10.1093/icb/45.1.172

Bang WY, Kim SW, Jeong IS, Koiwa H, Bahk JD (2008) The C-terminal region (640- 967) of *Arabidopsis* CPL1 interacts with the abiotic stress- and ABA-responsive transcription factors. Biochem Biophys Res Commun. 372: 907-912. doi: 10.1016/j.bbrc.2008.05.161

Behringer C, Bartsch K, Schaller A (2011) Safeners recruit multiple signalling pathways for the orchestrated induction of the cellular xenobiotic detoxification machinery in *Arabidopsis*. Plant Cell Environ 34: 1970-1985. doi: 10.1111/j.1365-3040.2011.02392.x

Benjamini, Y., and Hochberg, Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc 57, 289-300. doi: 10.2307/2346101

Bessede A, Gargaro M, Pallotta MT, Matino D, Servillo G, Brunacci C, et al. (2014) Aryl hydrocarbon receptor control of a disease tolerance defence pathway. Nature 511:184–90. doi:10.1038/nature13323

Brazier-Hicks M and Edwards R. (2005) Functional importance of the family 1 glucosyltransferase UGT72B1 in the metabolism of xenobiotics in *Arabidopsis thaliana*. Plant J 42: 556-566. doi: 10.1111/j.1365-313X.2005.02398.

Calabrese D, Bille J, Sanglard D (2000) A novel multidrug efflux transporter gene of the major facilitator superfamily from *Candida albicans* (*FLU1*) conferring resistance to fluconazole. Microbiol 146: 2743-2754. doi: 10.1099/00221287-146-11-2743

Chen J, Chen B, Zhang D (2015) Transcript profiling of *Populus tomentosa* genes in normal, tension, and opposite wood by RNA-seq. BMC Genomics 16: 164. doi: 10.1186/s12864-015-1390-y

Chou HH and Holmes MH (2001) DNA sequence quality trimming and vector removal. Bioinformatics 17:194-198. doi: 10.1093/bioinformatics/17.12.1093

Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 21, 3674–3676. doi: 10.1093/bioinformatics/bti610

Di Baccio D, Galla G, Bracci T, Andreucci A, Barcaccia G, Tognetti R, and Sebastiani L (2011) Transcriptome analyses of *Populus* × *euramericana* clone I-214 leaves exposed to excess zinc. Tree Physiol. 31 (12): 1293-1308. doi: 10.1093/treephys/tpr106

Dua M, Singh A, Sethunathan N and Johri AK (2002) Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 59, 143–152. doi: 10.1007/s00253-002-1024-6

Ekman DR, Wolfe NL, Dean JF (2005) Gene expression changes in *Arabidopsis* thaliana seedling roots exposed to the munition hexahydro-1,3,5-trinitro-1,3,5- triazine. Environ Sci Technol 39, 6313-6320. doi: 10.1021/es050385r

Falgueras J, Lara AJ, Fernández- Pozo N, Cantón FR, Pérez- Trabado G, Claros G (2010) SeqTrim: a high-throughput pipeline for pre-processing any type of sequence read. BMC Bioinformatics. doi: 10.1186/1471-2105-11-38

Fekete Z, Rajnai Z, Nagy T, Jakab KT, Kurunczi A, Gémes K, Herédi-Szabó K, Fülöp F, Tóth GK, Czerwinski M, Loewen G, Krajcsi P (2015) Membrane Assays to characterize interaction of drugs with ABCB1. J Membr Biol 248(6):967-77. doi: 10.1007/s00232-015-9804-y.

Fode B, Siemsen T, Thurow C, Weigel R, Gatz C (2008) The Arabidopsis GRAS protein SCL14 interacts with class II TGA transcription factors and is essential for the activation of stress-inducible promoters. Plant Cell 20: 3122- 3135. doi: 10.1105/tpc.108.058974

Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inze D, Mittler R. and Van Breusegem F (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiol 141, 436-445. doi: 10.1104/pp.106.078717

Gaedeke N, Klein M, Kolukisaoglu U, Forestier C, Muller A, Ansorge M, Becker D, Mamnun Y, Kuchler K, Schulz B, Mueller-Roeber B, and Martinoia E (2001) The Arabidopsis thaliana ABC transporter AtMRP5 controls root development and stomata movement. EMBO J 20:1875 -1887. doi: 10.1093/emboj/20.8.1875

González-Gayte I, Bautista-Moreno R, Claros MG (2015) DEgenes hunter a self-customised gene expression analysis workflow for non-model organisms. Bioinformatics and Biomedical Engineering. Tools for Next Generation Sequencing Data Analysis. 2, 313-321. doi: 10.1007/978-3-319-16480-9_31

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J et al (2013) *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc 8,1494–1512. doi: 10.1038/nprot.2013.084

Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol **Cell** Biol 8(10): 774-85. doi: 10.1038/nrm2249

Hayashi K, Schoonbeek H., De Waard MA (2002) Bcmfs 1, a novel major facilitator superfamily transporter from *Botrytis cinerea*, provides tolerance towards the natural toxic compounds camptothecin and cercosporin and towards fungicides. Appl Environ Microb 68: 4996-5004. doi: 10.1128/AEM.68.10.4996-5004.2002

Huang G and Elferink CJ (2012) A novel nonconsensus xenobiotic response element capable of mediating aryl hydrocarbon receptor-dependent gene expression. Mol. Pharmacol. 81, 338–347. doi: 10.1124/mol.111.075952.

Jansson S and *Douglas* CJ (*2007*) Populus: a model system for *plant biology.* Annu Rev **Plant** Biol *58, 435-458.* doi: 10.1146/annurev.arplant.58.032806.103956

Jörnvall H, Persson B, Krook M, Atrian S, González-Duarte R., Jeffery J and Ghosh D (1995) Short-chain dehydrogenases/reductases (SDR). Biochem 34(18), 6003-6013. doi: 10.1046/j.1432-1033.2002.03130.x

Kavanagh KL, Jörnvall H, Persson B, Oppermann U (2008) Medium- and short-chain dehydrogenase/reductase gene and protein families: the SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. Cell Mol Life Sci. 65(24):3895-906. doi: 10.1007/s00018-008-8586-0

Langmead B and Salzberg S (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods. 9:357-359. doi: 10.1038/nmeth.1923

Leigh MB, Prouzova P, Mackova M, Macek T, Nagle DP, Fletcher JS (2006) Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. Appl Environ Microbiol 72, 2331-2342. doi:

Li S and Chou HH (2004) LUCY2: an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics 20:3657-3665. doi: 10.1093/bioinformatics/bth302

Love MI, Huber W and Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15, pp. 550. doi: 10.1186/s13059-014-0550-8

Lüchmann KH, Clark MS, Bainy AC, Gilbert JA, Craft JA, Chipman JK, Thorne MAS, Mattos JJ, Siebert MN, Schroeder DC (2015) Key metabolic pathways involved in xenobiotic biotransformation and stress responses revealed by transcriptomics of the mangrove oyster *Crassostrea brasiliana*. Aquat Toxicol. 166, 10-20. doi: 10.1016/j.aquatox.2015.06.012

Martin JA and Wang Z (2011) Next-generation transcriptome assembly. Nature Rev Genet 12:671–682. doi:10.1038/nrg3068

McMillan BJ and Bradfield CA (2007) The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. Molecular Pharmacology 72: 487-498. doi: 10.1124/mol.107.037259

Murashige T and Skoog F (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiol Plant *15 (3): 473–497.* doi: 10.1111/j.1399-3054.1962.tb08052.x

Mylona PV, Polidoros AN, Scandalios JG (2007) Antioxidant gene responses to ROS-generating xenobiotics in developing and germinated scutella of maize. J Exp Bot 58(6): 1301-1312. doi: 10.1093/jxb/erl292

Nueda MJ, Tarazona S, Conesa A (2014) Next maSigPro: updating maSigPro Bioconductor package for RNA-seq time series. Bioinformatics. 30(18):1-5. doi: 10.1093/bioinformatics/btu333

O'Sullivan LA and Mahenthiralingam E (2005) Biotechnological potential within the genus Burkholderia. Under the Microscope. Lett Appl Microbio. 41, 8-1. doi: 10.1111/j.1472-765X.2005.01758.x

Olsen L, Oostenbrink C, Jørgensen FS (2015) Prediction of cytochrome P450 mediated metabolism. Adv **Drug** Deliv Rev 23(86): 61-71. doi: 10.1016/j.addr.2015.04.020

Pao SS, Paulsen IT, Saier MH Jr (1998) Major facilitator superfamily. Microbiol Mol Biol Rev 62: 1–34. doi: 10.9783/9781512806809-002

Petersen RA, Niamsup H, Berenbaum MR and Schuler MA (2003) Transcriptional response elements in the promoter of CYP6B1, an insect P450 gene regulated by plant chemicals. Biochim Biophys Acta 1619, 269–282 doi: 10.1016/S0304-4165(02)00486-5

Prado-Lopez S, Conesa A, Armiñán A, Martínez-Losa M, Escobedo-Lucea C, Gandia C, Tarazona S, Melguizo D, Blesa D, Montaner D et al (2010) Hypoxia promotes efficient differentiation of human embryonic stem cells to functional endothelium. Stem Cells 28: 407- 418. doi: 10.1002/stem.295.

Proudfoot N, Furger A, Dye M (2002) Integrating mRNA processing with transcription. Cell 108: 501-512. doi: 10.1016/S0092-8674(02)00617-7

Ramel F, Sulmon C, Cabekki-Hurtadi F, Magniette ML, Renou JP, Amrani A, Couée I, Gouesbet G (2007) Genome-wide interacting effects of sucrose and herbicide-mediated stress in *Arabidopsis thaliana*: novel insights into atrazine toxicity and sucrose-induced tolerance. *BMC Genomics* 8:450. doi: 10.1186/1471-2164-8-450

Ramel F, Sulmon C, Serra AA, Gouesbet G, Coueé I (2012) Xenobiotic sensing and signalling in higher plants. J Exp Bot 1-16. doi: 10.1093/jxb/ers102.

Reddy VS, Shlykov MA, Castillo R, Sun EI, Saier MH (2012) The major facilitator superfamily (MFS) revisited. BMC Bioinformatics. 11: 38. doi: 10.1111/j.1742-4658.2012.08588.x

Rizza S, Conesa A, Juarez J, Catara A, Navarro L, Duran-Vila N, Ancillo G (2012) Microarray analysis of Etrog citron (Citrus medica L.) reveals changes in chloroplast, cell wall, peroxidase and symporter activities in response to viroid infection. Mol Plant Pathol 13(8): 852-64. doi:10.1111/j.1364-3703.2012.00794.x

Robinson MD, McCarthy DJ, and Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139-140. doi:10.1093/bioinformatics/btp616.

Scheetz TE, Trivedi N, Roberts CA, Kucaba T, Berger B, Robinson NL, Birkett CL, Gavin AJ, O'Leary B, et al (2003) ESTprep: preprocessing cDNA sequence reads. Bioinformatics 19: 1318-1324. doi:10.1093/bioinformatics/btg159

Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassman M, Lightfoot S, Menzel W, Granzow M, Ragg T (2006) The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 7:3. doi: 10.1186/1471-2199-7-3

Seto M, Kimbara K, Shimura M, Hatta T, Fukuda M, Yano K (1995) A Novel Transformation of Polychlorinated Biphenyls by *Rhodococcus sp*. Strain RHA1. Appl Environ Microbiol 61(12): 4510-4513. doi: 10.1128/aem.61.9.3353-3358.1995

Shannon P, Markiel A, Ozier O (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11): 2498-504. doi: 10.1101/gr.1239303

Shiquan Wu and Jing Li (2007) Comparative analysis of gene-coexpression networks across species. ISBN 978-3-540-72030-0. ISBRA LNBI 4463: 615-626. doi:10.1007/978-3-540-72031-7_56

Singh K, Foley RC, Oñate-Sanchez L (2002) Transcription factors in plant defense and stress responses. Curr Opin **Plant** Biol 5: 430-436. doi: 10.1016/S1369-5266(02)00289-3

Smyth G (2005) Limma: linear models for microarray data. In Bioinformatics and Computational Biology Solutions using R and Bioconductor, G. R., C. V., D. S., I. R., and H. W., eds. (New York, Springer). 397-420. ISBN 978-0-387-29362-2

Tarazona S, García-Alcalde F, Dopazo J, Ferrer A, Conesa A (2011) Differential expression in RNA-seq: a matter of depth. Genome Res. 21(12):2213-23. doi: 10.1101/gr.124321.111

Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell 143: 606–616. doi: 10.1016/j.cell.2010.10.020.

Ulrich RG (2003) The toxicogenomics of nuclear receptor agonists. **Curr** Opin Chem Biol 7: 505–510. doi: 10.1016/S1367-5931(03)00080-2

Wang Y, Dou D, Wang X, Li A, Sheng Y, Hua C, Cheng B, Chen X, Zheng X, Wang Y (2009) The PsCZF1 gene encoding a C2H2 zinc finger protein is required for growth, development and pathogenesis in *Phytophthora sojae.* Micro Path 47: 78-86. doi: 10.1016/j.micpath.2009.04.013.

Wang Y, Peng X, Xu W, Luo YB, Zhao W, Hao J, Liang Z, Zhang Y, Huang KL (2012) Transcript and protein profiling analysis of OTA-induced cell death reveals the regulation of the toxicity response process in *Arabidopsis thaliana*. J Exp Bot 63: 2171–2187. doi: 10.1093/jxb/err447.

White JR, Roberts M, Yorke JA (2008) Figaro: a novel statistical method for vector sequence removal. Bioinformatics. 24:462-467. doi: 10.1093/bioinformatics/btm632

Yang ZB, Eticha D, Albacete A, Rao IM, Roitsch TWJ (2012) Horst Physiological and molecular analysis of the interaction between aluminium toxicity and drought stress in common bean (*Phaseolus vulgaris*). J Exp Bot 63(8): 3109-3125. doi: 10.1093/jxb/ers038

Zechmann B (2014) Compartment-specific importance of glutathione during abiotic and biotic stress. Front **Plant** Sci 5:566. doi: 10.3389/fpls.2014.00566

Zhang W, Chu Y, Ding C, Zhang B, Huang Q, Hu Z, Huang R, Tian Y, Su X (2014) Transcriptome sequencing of transgenic poplar (*Populus* × *euramericana* ‘Guariento’) expressing multiple resistance genes. BMC Genet 15(Suppl 1):S7. doi: 10.1186/1471-2156-15-S1-S7