MICROARRAY ANALYSIS OF ESTRADIOL-REGULATED GENES IN ARABIDOPSIS THALIANA SEEDLINGS

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Kazeem Ademola Adetunji entitled "Microarray Analysis of Estradiol-regulated Genes in *Arabidopsis thaliana* Seedlings." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Biology.

Camelia Maier, Major Professor

We have read this thesis and recommend its acceptance:

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Accepted:

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ABSTRACT

KAZEEM ADEMOLA ADETUNJI

MICROARRAY ANALYSIS OF ESTRADIOL-REGULATED GENES IN ARABIDOPSIS THALIANA SEEDLINGS

AUGUST 2012

Steroids are well-known regulators of physiological processes in plants and animals. The only known plant steroid hormones are brassinosteroids. Earlier studies demonstrated that animal steroid hormones have signaling activities in plants. The goal of this study was to determine the regulatory effects of estradiol on Arabidopsis gene expression. Seedlings were treated with 10 nM 17β-estradiol for three hours at the end of the seven-day cultures in liquid Murashige and Skoog medium. RNA was isolated from estradiol-treated and control plants and used for microarray analyses. From a total of 212 selected genes, 35% were up-regulated, 52% down-regulated and 13% non-responsive to estradiol treatment. Gene expression was altered mostly for secondary metabolism and CYP450 genes. Stress related genes were up-regulated indicating a possible synergistic effect of estradiol in plant growth and stress responses. Estradiol did not affect brassinosteroid biosynthesis genes. This study will help understand the steroid hormone and xenobionts effects on plants.

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ABBREVIATIONS

BA - Benzyladenine

BL - Brassinolide

BR – Brassinosteroid

Col – Colombia, Arabidopsis ecotype

DMSO- Dimethyl sulfoxide

dwf4 - Arabidopsis dwarf mutant

ER - Estrogen receptor

ERα - Estrogen receptor alpha

ERβ - Estrogen receptor beta

mER - Membrane estrogen receptor

MS - Murashige and Skoog medium

TAIR – The Arabidopsis Information Resource

wt – wild type

CHAPTER I

INTRODUCTION

Steroid Hormones in Animals and Plants

Sterols are ubiquitous and essential cellular components in all eukaryotes. They regulate cell membrane fluidity and permeability and are precursors of a vast array of bioactive compounds involved in important cellular and developmental processes, such as steroid hormones (Bishop and Yokota, 2001; Clouse, 2002). Steroids are a group of natural compounds regulating several critical physiological functions in animal and plant organisms (Golovatskaya, 2004). Steroidal hormones of higher animals include estrogens, androgens, progestins, mineralocorticoids and glucocorticoids. Estradiol is the major estrogen in vertebrates (Fig. 1). Also known as the female sexual hormone, it is found in males in trace amounts. Brassinosteroids (BRs) are the only known plant steroid hormones. The first biologically active plant BR was isolated from the pollen of rapeseed Brassica napus in 1979 (Grove et al., 1979). Brassinosteroids play essential roles in plant development (Bishop and Koncz, 2002; Janeczko et al., 2003; Deng et al., 2007). They are structurally related to animal and insect steroid hormones, and are found in all plant organs (Bajguz and Tretyn, 2003). Figure 1 shows the similarity in the chemical structures of 17beta-estradiol and brassinolide (BL).

Estradiol, like other steroid hormones in animals, is derived from cholesterol, which is converted to androstenedione, which in turn is converted to testosterone. Testosterone

is then aromatized to estradiol (Khryanin, 2002). Plant sterols are biosynthesized from mevalonic acid, through squalene-2,3-oxide and cycloartenol. Brassinolide is a derivative of BR and its biosynthesis begins with the reduction of campesterol to campestanol, which is oxidized to 6α-hydroxycampestanol and this to 6-oxocampestanol (Suzuki et al., 1995).

A B

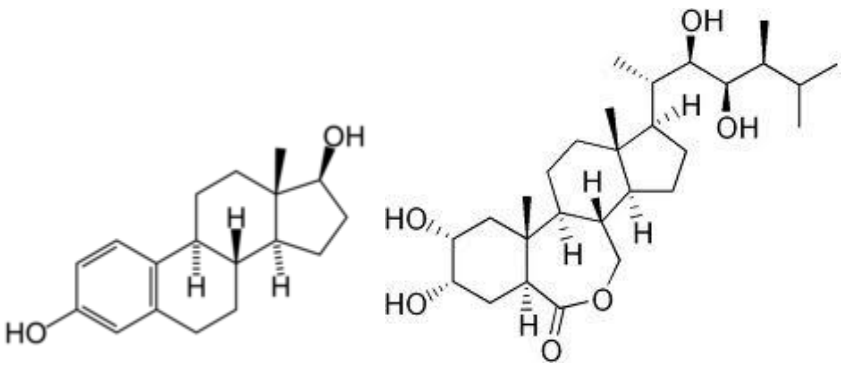

Figure 1. The chemical structure of 17beta-estradiol (A) and brassinolide (B) (Bajguz and Tretyn, 2003)

Estradiol acts as a signal molecule by interacting with transcriptional factors known as estrogen receptors. The three known types of estrogen receptors are ERα, ERβ, and mER (membranes ER) (Morito et al., 2001; Ropero et al., 2002; Prossnitz et al., 2008). The actions of 17β-estradiol at the membrane result from the binding to estrogen receptor (mER), which rapidly activate cellular signaling systems upon ligation. This binding

generates second messengers and has potential biological consequences in a variety of target cells (Kelly and Levin, 2001). Estradiol can also cross the cell membrane and interact with cytoplasmic estrogen receptors. After binding to a receptor in the cytoplasm, the receptor-ligand complex changes its structural conformation and is transferred into the nucleus where it binds to corresponding DNA response elements and triggers gene expression. Estradiol is responsible for the development and function of the female sexual organs in vertebrates and has modulatory functions on organs such as bone, liver, brain, heart and blood vessels, etc. in humans (Dimitrova et al., 2002).

In plants, BR is recognized by a plasma membrane-bound receptor known as BRI1. The BRI1 receptor is a typical receptor-like kinase with an extracellular domain interrupted by a 70-amino acid island domain and BRs bind at the island domain of the receptor (Kinoshita et al., 2005). Brassinosteroids are classified as C27, C28 or C29 compounds, based on the length of the side chain (Bajguz and Tretyn, 2003) and they have been known as growth-promoting steroid hormones that regulate multiple physiological and developmental processes in plants (Deng et al., 2007). Brassinosteroids have an important role in the light-induced development of plants (Li et al., 1996, Luo et al., 1998) accelerating cell elongation by affecting the cytoskeleton and cell wall structure (Sun et al., 2005).

Brassinosteroid deficiency is responsible for dwarfism in Arabidopsis plants by altering shoot development as a result of the inhibition of hypocotyl elongation (Salchert et al., 1998). Mutants in the biosynthesis and signaling of BL, the most bioactive BR, have been used to establish essential roles of BR in plant growth and development.

Biosynthesis mutants of BR respond to exogenous BR application whereas the signaling mutants generally do not (Bishop, 2003). Exogenously applied BL to Arabidopsis dwarf mutant dwf4 rescued the phenotype, which demonstrated that the dwarf phenotype is entirely BR dependent (Azpiroz et al., 1998).

Brassinosteroids are also known to have stress-ameliorative properties in plants. Recent research (Ali et al., 2008; Bajguz and Hayat, 2009) has revealed that BRs help plants cope with such stresses like heavy metals, water deficiency, salt, high and low temperatures, and pathogen attack. The ability of BRs to induce tolerance in plants to a broad spectrum of stress agents, results largely from the interaction with other phytohormones (Bajguz and Hayat, 2009; Kanwar et al., 2012).

The control of sex determination in plants is far less understood than that in animals. Most plants are hermaphrodites, with flowers containing both sex organs, stamens and carpels. A plant species in which male and female sexual organs are found in different flowers is known to be monoecious. Recent studies revealed that BRs are involved in controlling sex determination in monoecious maize by promoting masculinity of the male inflorescence. This finding suggests that in monoecious maize, BRs evolved to perform a sex determination function not found in hermaphrodite plants (Hartwig et al., 2011).

Steroid Hormone Interactions between Plants and Animals

A significant body of literature suggests that hormonal signaling systems are not restricted to certain animal or plant phyla (Miller and Heyland, 2010). Animals are able to acquire and use plant steroids for synthesis of endogenous hormones. For example,

sterols from plants are essential for the synthesis of ecdysteroids, a crucial group of insect morphogenic steroids (Miller and Heyland, 2010). It is even thought that some endogenous hormones, such as ecdysteroids in insects and thyroid hormones in echinoderms may have had exogenous origins (Miller and Heyland, 2010). Phytoecdysteroids, thought to be deterrents of insect herbivores in plants, are taken in by larvae and become insect ecdysteroid or molting hormones by a one-step chemical modification (Schoonhoven, et al., 1992). Insects are unable to synthesize sterols de novo, and depend on an intake of sterols or metabolic precursors from their diet.

The availability of phytoecdysteroids is a limiting step in hormone synthesis. Due to the importance of hormones in animal development and physiology, these organisms likely evolved mechanisms to detect the compounds or their precursors in the environment (Schoonhoven et al., 1992). Therefore we can gain insight into the evolution of hormonal signaling pathways and their relevance in animal life by studying the significance of exogenous hormone precursor sources.

Some non-steroidal secondary compounds produced by plant tissues possess estrogenic activity in animals and are known as phytoestrogens. Examples of phytoestrogens include subclasses of flavonoids, such as isoflavones, coumestans, and flavonols (Morito et al., 2001). Phytoestrogens mediate several physiological processes in animals via transcriptional regulation and intracellular signaling modulation through nuclear receptors and secondary messengers (Morito et al., 2001). Mammalian female reproduction, breast development, cardiovascular and neuroprotection processes, and cell

division are among the typical processes promoted by phytoestrogens (Prossnitz et al., 2008).

Animal steroid hormones were also detected in plants at the beginning of the twentieth century (Dohrn et al., 1926), and it was determined that their content changes depending on plant species, tissue, and phase of development (Erdal and Dumlupinar, 2011). Since then, research focused on two directions: the study of endogenous steroid hormone occurrence, activity and mechanism of action in plants and the study of the effects of mammalian steroid hormone applications to plants. It has been reported that, when applied on plants, steroid hormones affect different growth stages from germination to flowering (Janeczko and Skoczowski, 2005; Erdal and Dumlupinar, 2010; Pauli et al., 2010; Erdal and Dumlupinar, 2011; Janeczko, 2011). These researchers have reported that mammalian steroid hormones significantly stimulated plant growth and development especially at low concentrations. Comparative studies on the effect of the plant hormones gibberellic acid and benzyladenine (BA), as well as the animal sex hormones testosterone and estradiol, demonstrated that only estradiol and BA stimulate female sex expression in hemp plants (Khryanin, 2002). Also, this study shows that estradiol is able to regulate sex expression in certain plant species such as Quaking aspen, Populus tremuloides Michx. (Khryanin, 2002).

Plants possess the enzyme machinery for the anabolism of applied mammalian steroid hormones (Li et al., 1997). It has been shown that the conversion of applied cholesterol into pregnenolone, which is an important precursor to the synthesis of plant steroid aglycones with cardiotonic activity—cardenolides, occurs in the mitochondria of

foxglove, Digitalis sp. (Lindemann and Luckner, 1997). The best example of the structural and functional conservation of these processes is the reaction catalyzed by the steroid 5α reductase (5αR). It has been shown that the 5αR of plants and animals are orthologs and catalyze the same type of reaction in the biosynthesis of steroids (Li et al., 1997; Rosati et al., 2003). Animal steroid hormone progesterone and membrane progesterone-binding proteins are found in plants (Iino et al., 2007). Progesterone plays a role in the regulation of plant growth and development by binding to these proteins (Iino et al., 2007).

The Model Plant Arabidopsis thaliana

The easily manipulated, genetically tractable Wall cress or Mouse-ear cress, Arabidopsis thaliana, is a small annual plant in the mustard family (Brassicaceae) that has become the model system of choice for plant biology research (Huala et al., 2001). This plant has been the subject of study by an estimated 7000 researchers around the world (Huala et al., 2001). The 120-megabase genome of Arabidopsis is organized into five chromosomes and contains an estimated 20,000 genes (Meinke et al., 1998). In addition to the large body of genetic, physiological and biochemical data gathered from studying this plant, Arabidopsis was the first higher plant whose genome was completely sequenced (Huala et al., 2001; Yamada et al., 2003; Baerenfaller et al., 2008). The completion of its genome sequencing has enhanced the value of Arabidopsis as a model for plant biology and the analysis of complex organisms in general. Although Arabidopsis is not of major agronomic significance, it offers important advantages for basic research in genetics and molecular biology due to its relatively small genome,

extensive genetic and physical maps of its chromosomes, and a large number of available mutant lines and complete knockout library (Huala et al., 2001). With the knowledge gained from this established model plant as a reference system, scientists can move forward with more rapidly initiating improvements in plants of economic and cultural importance.

Microarray, the Technique of Choice for Studying Gene Expression

Microarray experiments have been widely used for studying gene expression. These are a class of biotechnologies that allow the monitoring of expression levels for thousands of genes. The microarray analysis examines complex biological interactions simultaneously within and between different probes, which are identified by specific locations on a slide array (Wisman and Ohlrogge, 2000). Arrays are hybridized in a series of experiments with probes derived from seeds, leaves, and roots of plant species such as Arabidopsis, rice, alfalfa, maize and others. Brassinosteroid-regulated genes in wild type Arabidopsis and in the BR-deficient mutant det2 were identified by employing microarray analysis (Goda et al., 2002). Gene expression changes are controlled through highly complex, non-linear interactions between proteins, DNA, RNA, and a variety of metabolites. Some of these changes will be of critical importance to the biological process of interest (either causal or directly consequential), while others may be peripheral or non-essential. Microarrays expression analysis has a number of features that have made it the most widely used method for profiling mRNA expression.

Microarray technology is the solution of choice for advancing plant, animal, and applied research (Lee, 2008). This technology will enable agricultural researchers and breeders to better understand animal and plant development, growth, and disease processes, which are critical to the crop improvement, meat, and dairy industries. They will also enable scientists utilizing model organisms to advance their understanding of biological processes and human disease.

Rationale, Goal, and Objectives of the Study

Earlier studies have demonstrated that animal steroid hormones have signaling activities in plants and that plant steroid-like compounds act through steroid receptors and/or through non-receptor mechanisms in animals, as detailed above (Janeczko and Skoczowski, 2005; Erdal and Dumlupinar, 2010; Pauli et al., 2010; Erdal and Dumlupinar, 2011; Janeczko, 2011). The mechanism of animal steroid signaling in plants is not well understood, however. In current years, research on the effects of xenoestrogens on both animals and plants has intensified due to the contamination of water and soil with different types of xenoestrogens. Studying the effects of chemically related exogenous signaling molecules on organisms of different kingdoms may result in the discovery of novel signaling systems. The presence of xenoestrogens in the environment has implications for understanding their hormonal function and mechanism of action in plants (Watson et al., 2005). Thus we can understand the link between the environmental factors and the regulation of the plant internal homeostatic systems for the purpose of practical application regarding crop improvement.

Chemically inducible expression systems have been developed to study gene functions. Among those are several vector systems regulated by estradiol (Zuo et al., 2000; Brand et al., 2006; Abraham et al., 2011). For an inducible expression system to be reliable, its activation should not have any non-specific physiological or other undesirable effects on the host.

In this study, 17β-estradiol was applied to Arabidopsis seedlings and microarray analysis was performed for the purpose of establishing a baseline/control activity of estradiol-regulated genes, which can be used as a reference for further research on the effect of xenoestrogens on plants. Also, this study will be useful in understanding physiological responses of Arabidopsis to estradiol applications, especially for research employing estradiol inducible expression systems.

The objectives of this study were to:

1) Screen for genes in Arabidopsis seedlings that respond rapidly to estradiol treatment by microarray analysis in order to gain insight into the mechanism of estradiol action at the molecular level;
2) Determine similarities and differences in gene expression induced by a plant steroid hormone, BL, from a previous study by Goda et al. (2002) and an animal steroid hormone, 17β-estradiol, applied to Arabidopsis seedlings in this study.

No prior reports of microarray analysis study on the effect of estradiol on the regulation of Arabidopsis genes were found in published literature.

CHAPTER II

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wild type Arabidopsis ecotype Columbia (Col-0) seeds were obtained from Lehle Seeds, Inc. (Round Rock, TX) and Murashige and Skoog (MS) medium from Sigma-Aldrich Inc. (St. Louis, MO). Seeds were sterilized in 95% alcohol solution followed by 10 % bleach. They were rinsed extensively with sterile distilled water to remove traces of bleach. One mg sterilized seeds replicate was inoculated into freshly prepared MS liquid media containing 5 mM KNO_3, 2.5 mM K_2HPO_4, $MgSO_4$, 1 mM $Ca(NO_3)_2$, 1mM MES, 50µM FeEDTA, macroelements, micronutrients, vitamins and 0.8 % sucrose, pH 5.7 (Murashige and Skoog, 1962). Arabidopsis seeds were incubated for germination in dark for 48 hours. For the rest of the experiments, the seedlings were grown in liquid MS medium on shaker at room temperature, and a light intensity of 7360 Luz in an ArabiSun light chamber for 5 more days.

Estradiol was dissolved in Dimethyl sulfoxide (DMSO) (Sigma-Aldrich Inc., St. Louis, MO) to obtain a 0.1M stock solution. The final concentration of estradiol used for the MS medium was 10 nM, same concentration of BL in DMSO used by Goda et al., (2002) in their experiments. The seedlings were treated with 10 nM of estradiol for 3 hours at the end of the 7-day culture after which they were immediately frozen in liquid

nitrogen and stored at -80°C for RNA isolation. Arabidopsis seedlings with DMSO only served as control.

RNA Extraction

RNA was extracted from pools of seedlings using the Trizol reagent from the Qiagen RNeasy midi kit (Invitrogen Inc., Carlsbad, CA). RNA samples were purified using Qiagen RNeasy MinElute Columns Kits to remove any phenol that might have been carried over from Trizol during extraction. RNA samples were quantified using an ND1000 NanoDrop spectrophotometer. RNA quality analysis was performed with a bio-analyzer to make sure that the RNA was of good quality for hybridization.

Hybridization Strategy and Data Analysis

The Microarray analysis was performed at the Microarray Facility Center of The Noble Foundation, Ardmore, OK, using the procedure described in the Affymetrix GeneChip expression analysis technical manual. Briefly, 10 µg of total RNA was converted to cDNAs by reverse transcription using a superscript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA). The cDNAs were transcribed in vitro to form biotinylated complementary RNAs (cRNA) using the bio-array high yield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). Twenty µg of each sample of labeled cRNAs were fragmented to lengths of roughly 35-200 bases in fragmentation buffer (40 mM Tris-acetate, pH 8.1, 100 mM potassium acetate, and 30 mM magnesium acetate) at 94°C for 35 min.

Ten µg of each samples' fragmented cRNA was hybridized to Affymetrix ATH1 array GeneChip containing 22,810 probe sets at 45°C with rotation for 16 h. A total of three arrays were used, one array per biological replicate for each treatment (estradiol vs. control).

The arrays were washed and stained with streptavidin phycoerythrin conjugate (Molecular Probes, Eugene, OR). The scanning was done with the GeneChip Scanner 3000 (Affymetrix Technical Manual) using the full Affymetrix Gene Chip® instrument system. Each array chip was normalized relative to 18S ribosomal gene and each gene was compared between the control and estradiol-treated seedlings. Data from the Gene Chip instrument system was analyzed using the Gene Chip® analysis software to identify genes by fold expression change. A Welch t-test was used to measure the evidence of a change in the expression level of a gene (Goda et al., 2002; Jorstad et al., 2007; Wang et al., 2007). Unregulated genes had t-statistics close to zero while the t-statistics of up- and down- regulated genes were at least 2-fold positive and negative, respectively. Post-normalization cutoff of two-fold up- or down-regulation was used for defining differential gene expression. Genes were classified to be up-regulated and down-regulated based on their fold differences values before and after signal amplification. Three independent experiments with different plant samples were performed and genes that showed the same response in all three experiments were considered estradiol-regulated genes. The final gene expression analysis consisted of selecting the highly regulated genes for each time point, treated vs. untreated, and checking the genetic

database TAIR (http://www.arabidopsis.org/tools) for confirmation of potential protein predictions and annotation (Huala et al., 2001).

Quantitative RT-PCR Analysis

Verification of expression of six selected genes was performed in triplicates by qRT-PCR analysis. Two µg each of total RNAs isolated from estradiol-treated seedlings were treated with DNase1 and were converted to cDNAs using a Super Script first Strand synthesis system (Invitrogen, Carlsbad, CA). Primers were selected in the same region that was used for the probe array design. Quantitative RT-PCR was performed using the gene specific primers in Table I and transcript abundance was analyzed. Each PCR reaction contained 100 ng cDNA and 25 µM of each of the primers. Amplification was carried out in 40 cycles, 95°C for 30 s, 50-62°C for 30 s and 72°C for 30 s. Quantification of relative gene expression was done by comparative Ct method (Pfaffi, 2001).

The 18S ribosomal RNA gene was used as an internal control and to normalize the values for transcript abundance. Each experiment was normalized relative to the median and the means and SEs of three experiments were calculated. Student t-test was used to determine statistical differences in transcript abundance.

Table 1

Gene Specific Primers Used to Test Expression Levels by qRT-PCR

Gene/Primer Name	Primer Sequences	Gene Function
At1g60750_80F	GCATGGGTCTCTCCGACTTTTAC	Auxin-induced
At1g60750_140R	AGGAGAGCGACGGCGTTA	protein
At1g77950_334F	ATGGCTCTCCAAATCAACGAA	MADS box
At1g77950_399R	TTGCTCAAGTTCCTCGACATTG	transcriptional factor
At5g13200_271F	GAGCCAGTCATCGGAATGTTC	ABA-responsive
At5g13200_332R	AGGTTACGTGCGACGGTTTC	protein
At1g27730_467F	GGTGCCACTACGAAGGAAACA	Salt-tolerance zinc
At1g27730_528R	CGCACCTTCGGAGTTGGA	finger protein
At1g13420_446F	ATGCGCTTAAAGTACCGTTGAAA	Steroid
At1g13420_550R	TTTCACGTTCCTGCACACGTA	sulfotransferase
At4g13770_1326F	CCCGGGAATGCGTCTTG	Cytochrome P450
At4g13770_1386R	GCTGAGGAGAAGGTTCGCATA	monooxygenase

In the Gene/Primer Name, F – forward primer and R – reverse primer

CHAPTER III

RESULTS AND DISCUSSION

Microarray Analysis of Estradiol-regulated Genes

The goal of this study was to describe global gene expression changes induced by estradiol treatment of Arabidopsis seedlings. The experiments were conducted to determine functional similarity between BR-regulated genes and genes regulated by estradiol treatment. Wild type Arabidopsis thaliana (Col) seedlings were exposed to 10 nM 17β-estradiol for three hours at the end of seven-day cultures in liquid MS medium for the purpose of detecting genes that respond to17 beta-estradiol relatively rapidly. Microarray analyses of three independent experiments were performed on the RNA extracted from Arabidopsis seedlings treated with estradiol and from control (untreated) seedlings. This was done in order to separate genes that are truly differentially expressed from stochastic changes and to define differential expression based on the consensus of the three experiments.

When comparisons were made between control and estradiol-treated Arabidopsis seedlings, from a total of 212 selected genes, 35% were up-regulated, and 52% genes were down-regulation as shown in Figure 2. Appendix A and B list all up-regulated and down-regulated genes, respectively, grouped by their target descriptions. Genes that showed no significant pre- and post-signal amplification fold differences were classified as non-responsive genes and are listed with their target descriptions in Appendix C.

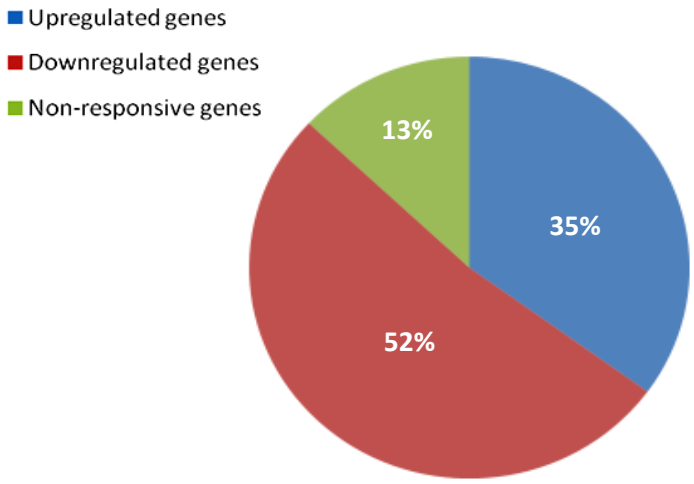

Figure 2. Frequencies of estradiol-regulated genes in wild type Arabidopsis seedlings. Distribution of up-regulated, down-regulated and non-responsive genes

Validation of Microarray Results by qRT-PCR Analysis

Verification of expression of six selected genes was performed in triplicates by qRT-PCR analysis. The genes were selected based on their functional interest. Auxin-induced protein, ABA-responsive protein and Steroid sulfotransferase were chosen for their plant hormonal functions. MADS box transcription factor gene was selected for its regulation of transcription and Salt–tolerance zinc finger protein gene was selected because the estrogen receptor is a zinc finger protein. Cytochrome p450 gene was selected for its implication in glucosinolate pathway in Arabidopsis. All gene expression data were consistent for both microarray and qRT-PCR. Statistically, the genes that were up-regulated in microarray analysis were also up-regulated in the qRT-PCR experiment and those that are down-regulated in microarray analysis experiments were also down-regulated in qRT-PCR as well.

Figure 3 shows the transcript abundance of three up-regulated genes namely, Auxin-induced protein (At1g60750), MADS box transcription factor (At1g77950), ABA-responsive protein (At5g13200) and one gene, salt-tolerance zinc finger protein (At1g27730), which was not affected by estradiol treatment.

The Auxin-induced protein (At1g60750) is an oxidoreductase from the aldo/keto reductase family (TAIR). The MADS box transcription factor (At1g77950) is a sequence-specific DNA binding transcriptional factor, type II subfamily of MADS box family and an Agamous-like transcriptional factor (AGL67) (NCBI). The ABA-responsive protein (At5g13200) is a GRAH domain-containing protein/ABA-responsive protein-related of unknown function (NCBI). The Salt-tolerance zinc finger protein (At1g27730) is a transcriptional factor involved in grolh, photosynthesis, photoprotection, and responses to environmental stress such as fungal infection, cold, oxidative and salt stresses (NCBI).

Auxin and ABA related genes were up-regulated by estradiol. It is possible that exogenous estrogens may play a role in regulating plant growth through a mechanism involving interaction with endogenous plant hormones. Estrone, at the concentration of 0.1g per plant stimulated the growth of Pisum sativum L. seedlings by about 40% and relatively new data for 1M estrogens stimulated the winter wheat seedling roots and leaves growing in vitro (Janeczko, 2005). The MADS box transcription factor (At1g77950) gene was up-regulated by estradiol. The MADS box transcription factor (At1g77950) involved in regulation of gene-specific transcription and cellular transcription. Further research can deduce a transcriptional control role for estradiol in Arabidopsis cellular metabolism activities.

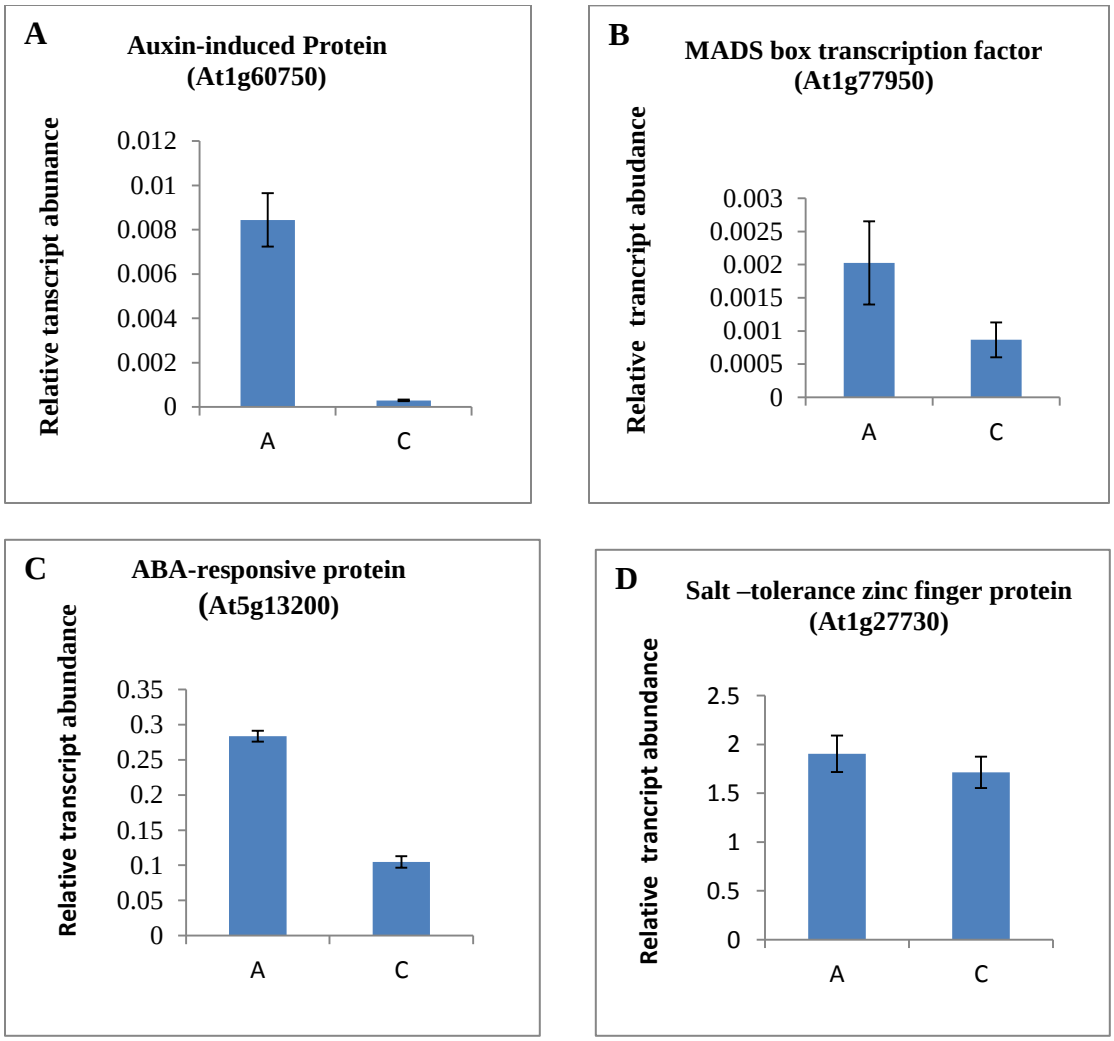

Figure 3. qRT-PCR results for selected estradiol up-regulated genes in wild type Arabidopsis seedlings as compared to control. A, Relative transcript abundance of Auxin-induced protein (At1g60750) compared with control. The p value was 0.01, which is below 0.05. Statistically shows significant difference between the two samples. B, Relative transcript abundance of MADS box transcription factor (At1g77950) compared with control. The p value was 0.09 which is higher than 0.05. This shows there was no statistical significance difference between the samples. C, Relative transcript of ABA-responsive protein (At5g13200) compared with control. The p value was 4.49973E-05 Indicating that there was significant difference between the two samples. D, Relative transcript of Salt-tolerance zinc finger protein (At1g27730) as compared with control. The p value was 0.24, which is higher than 0.05. This shows there was no statistical significance difference between the samples. Transcript abundance levels are presented as relative values that are normalized with respect to the levels of 18S ribosomal RNA. Data are means ± SD of three different plant samples. In the graphs A, experimental samples and C, control samples.

Two selected estradiol down-regulated genes confirmed by qRT-PCR are Steroid sulfotransferase (At1g13420) and Cytochrome P450 monooxygenase (At4g13770) as shown in Figure 4. The Steroid sulfotransferase (At1g13420) is a brassinosteroid sulfotransferase, ST4B (NCBI) involved in different aspects of hormone regulation (NCBI; Marsolais et al., 2007). The Cytochrome P450 monooxygenase (At4g13770) is an oxidoreductase acting on paired donors, with incorporation or reduction of molecular oxygen CYP83A1, which interacts selectively and non-covalently with oxygen and participates in pathways resulting in the synthesis of glucosinolates in Arabidopsis and other Brassicaceae (NCBI; Naur et al., 2003).

Student t-test was employed to determine the correlation between each sample with their control genes. The t-tests for Auxin-induced protein (At1g60750) and ABA-responsive protein (At5g13200) gene expression showed significant correlation confirming their up-regulation. The p values for Auxin-induced protein (At1g60750) as 0.01 and for ABA-responsive protein (At5g13200) was 4.49973E-05. The t-tests for Steroid sulfotransferase (At1g13420), Cytochrome P450 monooxygenase (At4g13770) gene expression also confirmed their down-regulation. The p value for Steroid sulfotransferase (At1g13420) was 0.02 and for Cytochrome P450 monooxygenase (At4g13770) was 0.04. The t-test for The MADS box transcription factor (At1g77950) and salt-tolerance zinc finger protein (At1g27730) shows no or little significant correlation between two samples. The p values for The MADS box transcription factor (At1g77950) and salt-tolerance zinc finger protein (At1g27730) were 0.09 and 0.24 respectively.

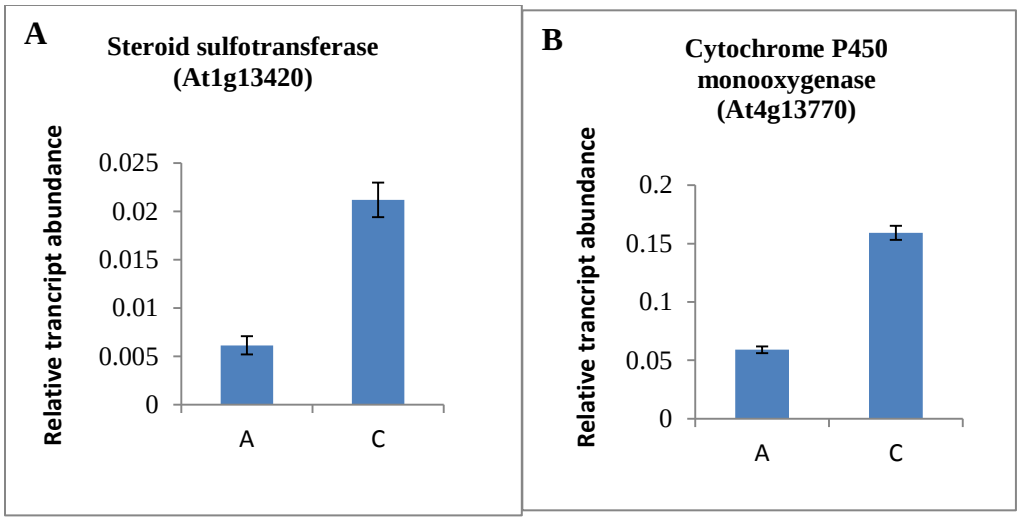

Figure 4**.** qRT-PCR results for down-regulated genes by estradiol in wild type Arabidopsis. A, Relative transcript abundance of Steroid sulfotransferase (At1g13420) compared with control. The p value was 0.02 showing a significant difference between the two samples. B, Relative transcript abundance of Cytochrome P450 monooxygenase (CYP83A1) (At4g13770) compared with control. The p value was 0.04, which is below 0.05. Statistically shows significant difference between the two samples. Transcript abundance levels are presented as relative values that are normalized with respect to the levels of 18S ribosomal RNA. Data are means ± SD of three different plant samples. A stands for the experimental samples and C stands for the control samples. In the graphs A, experimental samples and C, control samples.

Distribution of Estradiol-regulated Genes into Functional Categories

The estradiol-regulated genes are classified into functional categories based on their established or putative functions. Figure 5 shows the comparative distribution of up-regulated and down-regulated genes in Appendix A and B.

More transcriptional factor genes were down regulated than up-regulated. Among those, a putative heat shock transcription factor gene, At2g26150 was up-regulated, which correlates with more Heat and Stress Response genes being up-regulated and

anthocyanin2 gene, At1g56650, was down regulated, correlating to the down-regulation of the phenylpropanoid biosynthesis gene being also down-regulated.

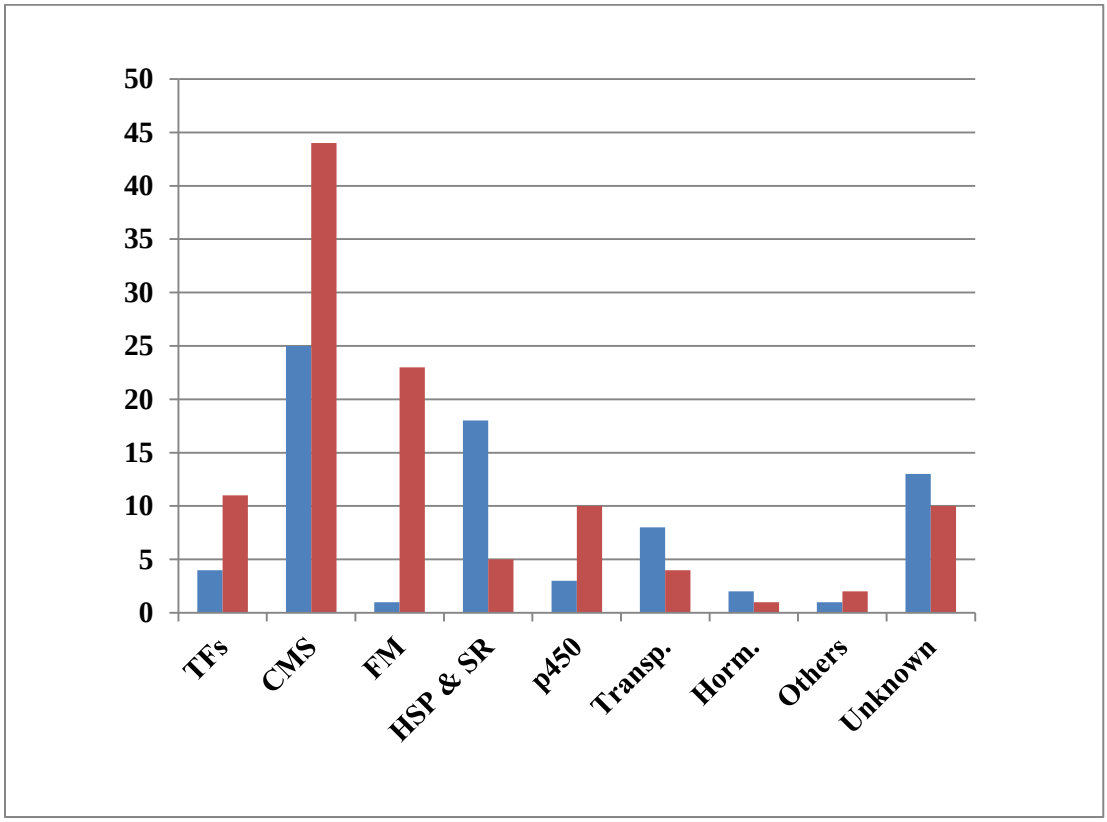

Figure 5. Distribution (actual number) of up-regulated (blue bars) and down-regulated (red bars) genes in Appendices A and B into functional categories based on their established or putative functions. TFs, Transcription Factors genes; CMS, Cellular Metabolism and Signaling genes; FM, Flavonoid Metabolism genes; HSP& SR, Heat shock protein and Stress Response genes; p450, p450 genes; Transp., Transporter genes; Horm., Hormone-related genes, Others, other genes not in the above categories; Unknown, genes with unknown functions.

Most of the gene transcripts regulated by estradiol are classified in the cellular metabolism and signaling functional category, both as up-regulated and down-regulated genes. Selective lists of these genes are presented in Tables 2 and 3. The results show that glutathione S-transferase genes are the most up-regulated genes.

Table 2

Selective List of Estradiol Up-regulated Genes in the Metabolism and Signaling Category in wild type Arabidopsis thaliana

Affymetrix no.	Gene description	Accession no.	Ratio(A/C)
267008_at	Putative ABC transporter	At2g39350	2.040027
250279_at	ABA-responsive protein	At5g13200	2.29497
264912_at	Auxin-induced protein	At1g60750	3.673437
246911_at	Transcription factor TINY	At5g25810	2.120924
258975_at	WRKY-like transcription regulator	At3g01970	2.238593
266267_at	Putative glutathione S-transferase	At2g29460	4.58978
254889_at	Osmotin precursor	At4g11650	2.48782
262349_at	Putative Lipid Transfer Protein	At2g48130	2.001653
266841_at	Putative heat shock transcription factor	At2g26150	2.168227
248125_at	2S storage protein	At5g54740	2.83782
253767_at	12S cruciferin seed storage protein	At4g28520	2.62575
263948_at	Putative harpin-induced protein hin1	At2g35980	3.29872
261242_at	Subtilisin-like serine protease	At1g32960	3.762862
262682_at	Anther-specific proline-rich-like protein	At1g75900	3.967695

Table 3

Selective List of Estradiol Down-regulated Genes in the Metabolism and Signaling Category in wild type Arabidopsis thaliana

Affymetrix	Gene description	Accession no.	Ratio(A/C)
266218_at	Putative cytochrome P450	At2g28850	0.186568
25938_at	Steroid sulfotransferase	At1g13420	0.251842
258851_at	Glutathione S-transferase	At3g03190	0.313985
255773_at	Flavonol 4'-sulfotransferase	At1g18590	0.453763
260451_at	Putative AP2 transcription factor	At1g72360	0.456729
266455_at	Putative bHLH transcription factor	At2g22760	0.458134
249890_at	Putative WRKY transcription factor	At5g22570	0.498516
247867_at	SNF1 related protein kinase	At5g57630	0.49699
256924_at	Anthocyanin 5-acyltransferase	At3g29590	0.414345
246522_at	bZIP DNA-binding protein	At5g15830	0.477736
262237_at	Acyl CoA thioesterase	At1g48320	0.488023
253697_at	Nucleotide pyrophosphatase	At4g29700	0.307751
250083_at	Putative glutathione S-transferase	At5g17220	0.326598
260547_at	Putative trypsin inhibitor	At2g43550	0.482253
254737_at	Fatty acid elongase	At4g13840	0.465455
245550_at	Cytochrome P450-like protein	At4g15330	0.490457
260385_at	Putative flavonol sulfotransferase	At1g74090	0.488541

A large number of Heat shock proteins (HSP) and stress genes such as Heat shock protein 101 (HSP101), Acidic endochitinase, Heat shock protein hsp70 and 17.6 kDa Heat shock protein (AA 1-156) were among those up-regulated by estradiol. The up-regulated Heat shock protein 21(At4G27670) is located in the chloroplast and is involved in response to such stresses as heat, high light intensity and hydrogen peroxide. The location of Heat shock protein 21(At4G27670) in the chloroplast as well as the involvement in the high light intensity might gives estradiol a regulatory roles in photosynthesis processes in plants. Among the few Heat shock proteins that were down-regulated by the estradiol treatment, At4g11190 is located in the endomembrane system and is involved in defense responses and lignin biosynthesis.

There were eight transporter genes that were up-regulated. Ammonium transport protein (At4g13510) was among up-regulated genes and is responsible for ammonium transport. Only four transporter genes that are all putative proteins were down-regulated. Sugar transporter like protein (At4g36670) is one of them and is involved in carbohydrate transmembrane transport.

ABA-responsive protein-like ABA-responsive protein (At5g13200) and Auxin-induced protein (At1g60750, with oxidoreductase activity) were the up-regulated hormone related genes while Putative protein myrosinase-binding protein-like (At5g35940) was the only down-regulated hormone related gene.

There were twenty-eight non-responsive genes that came across most of the functional classification of genes. Some of these non-responsive genes are Transcription factor genes such as putative protein transcription factor OBF3.1 (At4g18650) and

MADS box transcription factor GI: 1905943 (At1g77950). Salt-tolerance zinc finger protein (At1g27730) and putative cysteine proteinase inhibitor B (At2g31980) were among non- responsive genes when Arabidopsis seedlings treated with estradiol.

Down-regulation of P450s and Phenylpropanoid Biosynthetic Pathway

Pathway data are useful and powerful ways to summarize the network of molecular interactions induced by estradiol treatment of seedlings. A large number of the phenylpropanoid biosynthesis pathway genes were down-regulated by estradiol treatment suggesting that exogenous estradiol was capable of feed-back inhibiting enzyme activities of this pathway. The 4- and 5-hydroxylations of phenolic compounds in plants are catalyzed by cytochrome P450 enzymes in the phenylpropanoid biosynthetic pathway (Fig. 6) (Schoch et al., 2001). Down-regulation of cytochrome P450 genes as a result of estradiol treatment on Arabidopsis affects biosynthesis of phenylpropanoids. Future studies should focus on biochemical analyses of flavonoids in Arabidopsis plants under the estradiol treatment. The Cytochrome P450 monooxygenase (At4g13770) is an oxidoreductase acting on paired donors, with incorporation or reduction of molecular oxygen CYP83A1, which interacts selectively and non-covalently with oxygen and participates in pathways resulting in the formation of glucosinolates in Arabidopsis and other Brassicaceae (NCBI; Naur et al., 2003). Cytochrome-P450-dependent mono-oxygenases are involved in the biosynthesis of the glucosinolate core structure acting as catalysts (Wittstock and Halkier, 2002). It catalyzes enzymatic processes that convert aminoacids via an aldoxime to a reactive intermediate that is then conjugated with a

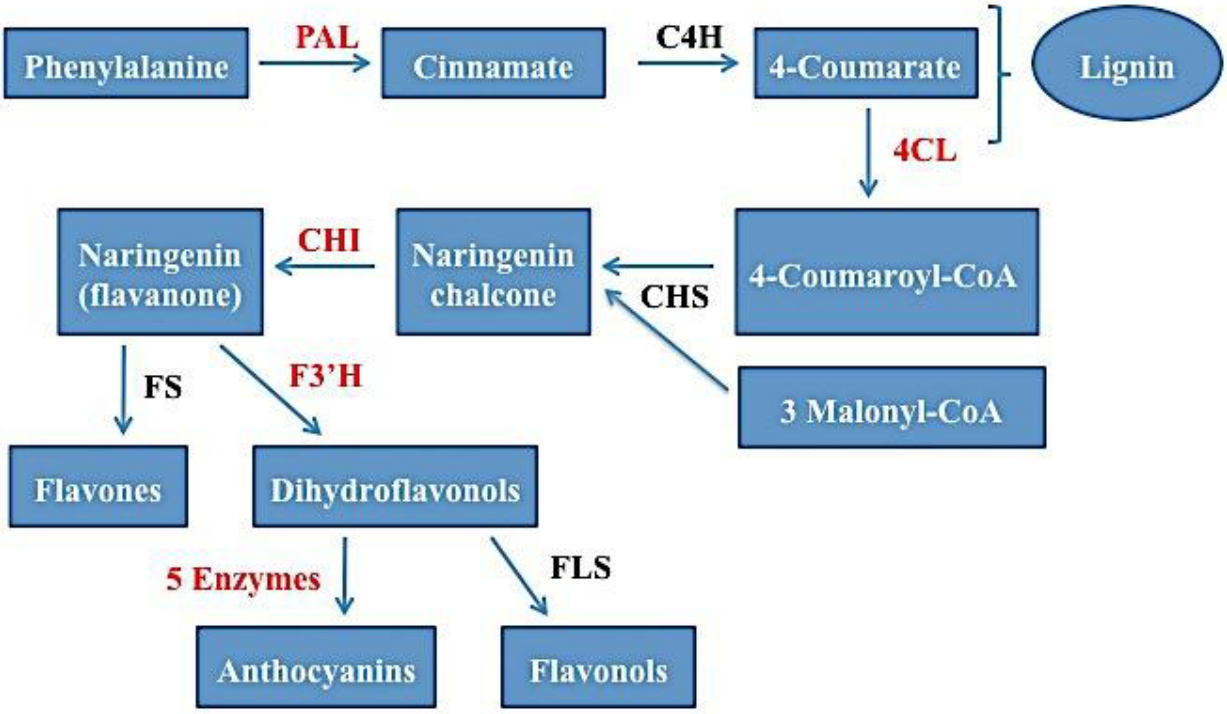

Figure 6. Phenylpropanoid biosynthesis pathway in Arabidopsis thaliana. Enzyme names are abbreviated as follows: PAL, phenylalanine ammonia lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaroyl:CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3'H, flavonoid 3' hydroxylase; FS, flavone synthase; FLS, flavonol synthase; 5 Enzymes, DFR – dihydroflavonol 4-reductase. Down-regulated genes by estradiol are indicated in red. (Adapted from Winkel-Shirley, 2001; Yin et al., 2012; Li et al., 2010)

sulfur donor for the synthesis of the glucosinolate core structure. The exogenous estradiol application to Arabidopsis seedlings might cause inhibition to the catalytic processes along the glucosinolates biosynthesis pathway that resulted in the down-regulation of Cytochrome P450 monooxygenase (At4g13770). The degradation products of glucosinolates play an important role in Arabidopsis plant defense against herbivores (Wittstock and Halkier, 2002).

Down-regulation of cytochrome P450 genes as a result of estradiol treatment on Arabidopsis inhibits the 4- and 5-hydroxylations of phenylalanine, cinnamate, and naringenin compounds in the pathway (Fig. 6). Down-regulated Dihydroflavonol 4-reductase (At5g42800) which involves in the F3H and F3'H enzymes activities as a result of estradiol treatment on Arabidopsis inhibiting the anthocyanin biosynthesis in the pathway. Down-regulation of Ferulate-5-hydroxylase (FAH1)(At4g36220) which involves in PAL (phenylalanine ammonia lyase), C4H (cinnamic acid 4-hydroxylase) biosynthesis inhibiting the lignin biosynthesis pathway.

Comparison Between Gene Regulation in BL-treated and Estradiol-treated Arabidopsis Seedlings

An earlier microarray analysis of gene expressions in Arabidopsis after treatment with BL, the only steroid hormone in plants, was published by Goda et al. (2002). After comparing the results obtained with estradiol treatment to those obtained by Goda et al., the conclusion was that no identical genes were affected by both treatments. However,

some genes with similar functions were either down-regulated or up-regulated in both treatments. Most of the up-regulated genes by BL in Goda et al. (2002) study, whose description were located in TAIR, were an auxin transcriptional factor, an auxin SAUR gene, cell wall organization and metabolism genes that respond to auxin stimulus, CytP450 genes involved in oxidation-reduction processes and response to wounding and a CytP450 gene whose protein serves as a control point between multiple photoreceptor systems and BR signal transduction (TAIR). The up-regulated genes designated by Goda et al. (2002) can be classified in four functional categories: cell-wall type genes, P450 monooxygenases, SAUR gene family and others. Down-regulated genes by BL treatment were genes on the BR biosynthetic pathway as well as the BRI1 gene, aminoacid and potassium transporters, a fatty acyl CoA reductase homolog, a 4-hydroxyphenylpyruvate dioxygenase, several P450 monooxygenase and cytochrome P450 genes, MYB-like genes, some auxin-inducible genes, two genes on the phenylpropanoid pathways among others (Goda et al., 2002).

No Heat shock proteins genes were affected by BL treatment compared to estradiol treatment. Arabidopsis thaliana seedlings treatments with BL and estradiol down-regulated several P450 genes such as Cytochrome p450 genes. However, the specific P450 genes down-regulated with BL treated are different than those down-regulated by estradiol. P450 genes in the CYP85 and CYP90 families that contribute to BR biosynthesis were down-regulated by exogenous BL but their expression was not changed by the estradiol treatment. Estradiol up-regulated the CYP710A1 gene, which encodes a protein with C22-sterol desaturase activity, shown to catalyze the conversion

of β-sitosterol to stigmasterol (plant sterols), but not that of 24-epi-campesterol to brassicasterol on the BR biosynthetic pathway (TAIR). It seems that estradiol application did not affect the expression of genes of the BR biosynthetic pathway in Arabidopsis. More so, the concentration of the estradiol used for treatment might be too small to cause any changes in the expression of BR biosynthetic enzymes. More research needs to be performed with different concentration of estradiol applied to different stages in Arabidopsis development to determine possible effects on the BRs biosynthetic pathway and signaling.

The P450 genes such as CYP83A1, PF00067 and H71417 were down-regulated by the estradiol treatment and not by the BL treatment. Table 4 summarizes the genes with similar functions that were down-regulated by both BL and estradiol treatments. Four Cytochrome p450 genes with similar functions were down-regulated in both treatments. The function of the down-regulated At3g45700 gene by estradiol is similar to that of At1g77380 in BL-treated seedlings in transporting amino acids and oligopeptides in the cells. There were two pairs of down-regulated MYB transcriptional factor genes by both treatments with similar functions in both treatments (Table 4), a pair of which is involved in anthocyanin biosynthetic branch of the phenylpropanoid pathway. Application of BL down-regulated only the Chalcone isomerase gene whose product catalyzes the conversion of chalcones into flavanones in the phenylpropanoid pathway. Estradiol down-regulated most of the genes on that pathway (Fig. 6). Both BL and estradiol treatment down-regulated Cinnamyl-alcohol dehydrogenase genes involved in lignin

biosynthesis and other oxidation-reduction processes in the cells. No glucosinolate pathways genes were affected by BL treatment compared to the estradiol treatment.

Table 4

Functional Similarity Between Genes Down-regulated in BL-treated and Estradiol-treated Arabidopsis thaliana Seedlings

Genes down-regulated in BL treatment	Genes down-regulated in Estradiol treatment	Common functions
AL035601 (At4g37370)	At5g42580	Cytochrome P450; heme binding, iron ion binding
X67816 (At4g37980)	At4g13770	Cytochrome P450; oxidoreductase activity
AF069495 (At4g39950)	At1g16410	Cytochrome P450; glucosinolate biosynthetic process
AF047834 (At1g06560)	At4g13770	Cytochrome P450; methyltransferase activity
X77499 (At1g77380)	At3g45700	Aminoacid/oligopeptide transporter activity
AF176000 (At4g01680)	At5g61420	MYB transcriptional factors; regulation of transcription
Y12776	At4g21020	Late embryogenesis-abundant (LEA) protein; embryo development
M86358	At3g55120	Chalcone isomerase; conversion of chalcones into flavanones (phenylpropanoid pathway)
X67816	At1g66800, At3g19450	Cinnamyl-alcohol dehydrogenase; lignin biosynthesis and oxidation-reduction processes
Z68157 (At1g71030)	At1g56650	Putative MYB domain transcriptional factors; anthocyanin biosynthetic process

The up-regulated functionally similar genes by both treatments are listed in Table 5. The P450 genes (At1g76690, At3g14660) up-regulated by estradiol treatment have similar functions as At2g27690 up-regulated by BL in Goda et al. (2002) study. They are all Cytochrome P450 genes and their function includes oxidation-reduction activity and response to wounding.

Table 5

Functional Similarity Between Genes Up-regulated in BL-treated and Estradiol-treated Arabidopsis thaliana Seedlings

Genes up-regulated in BL treatment	Genes up-regulated in BL treatment	Common functions
AC005824 (At2g27690) P450 monooxygenase	At1g76690, At3g14660	Oxidation-reduction, response to wounding
AL030978 Calcium-binding protein homolog	At1g21550 Calcium-binding EF-hand family protein	Calcium ion binding

A pair of calcium ion binding genes were up-regulated by both BL and estradiol treatments. Most of the hormonal-related genes up-regulated by BL were auxin-responsive genes, such as U18407 (At1g15580), S70188 (At1g04680) and U18406 (At1g04240), while estradiol up-regulated only At5g13200. Most of the up-regulated hormonal-related genes by estradiol were ABA, jasmonic acid, and salicylic acid-responsive genes.

Brassinolide treatment up-regulated a high percentage of very specific cell wall type genes functioning in cell growth and remodeling of cell walls (Goda et al., 2002), such as

xyloglucan endotransglycosylases, pectin esterases, acetylesterases and methylesterases, endoxyloglucan transferases, expansins, extensins, and arabinogalactan proteins. Estradiol treatment up-regulated few genes that are involved in cell growth, such as transcriptional factor TINY (At5g25810) involved in multidimensional cell growth and Organ size related 1 gene (At2g41230), and ER-localized plant hormone-responsive gene involved in post-embryonic organ development, regulation of cell division, and cell growth. It seems that BL directly affects cell wall production and remodeling while estradiol is more involved in organ development.

Conclusion and Significance

Microarray analysis corroborated by qRT-PCR showed that gene expression is triggered by estradiol, which is a xenobiont for Arabidopsis thaliana, since the plant does not synthesize it. Estradiol treatment did not affect genes on the BL biosynthesis pathway, or BL receptor gene. Estradiol had a strong effect in down-regulating genes on the phenylpropanoid and glucosinolate pathways, both very important secondary metabolite pathways in Arabidopsis. Contrary to BL, estradiol up-regulated HPSs involved in stress responses induced by many environmental factors. In conclusion, estradiol and BL do not share the same signaling mechanism in Arabidopsis.

Microarray technology produces vast quantities of data, as shown in the present study. These results allow generation of new insights into relevant biological information and hypotheses that will guide further research. Further analysis of the functions of the estradiol-regulated genes will provide insights into steroid and xenosteroid mechanisms

of action and will facilitate understanding of steroid hormone functions in plants. Techniques such as hierarchical clustering and self-organizing maps will be applied to the analysis of the data across multiple experiments. This study provides a baseline/control activity of estradiol-regulated genes in Arabidopsis seedlings, which can be used as a reference for further research on the effect of xenoestrogens on plants and can be replicated at different stages in plant development for the above purpose. Also, this study will be useful in understanding physiological responses of Arabidopsis to estradiol applications, especially for research employing estradiol inducible expression systems.

An area of follow-up research is to analyze promoter sequences of regulated transcripts to identify elements that may be involved in transcriptional regulation by a) looking up previously characterize elements in the promoters of transcripts that appear to be regulated by a known transcription factor (known sequence motifs can be located in promoter sequences using a variety of sequence search and alignment tools, such as those provided by NCBI, GenBank, RefSeq, and EPD, Eukaryotic Promoter Database) and b) searching for novel motifs in the promoters of transcripts that appear to be co-regulated (more challenging task, especially if the putative transcription factor is unknown) by using EPD and TRANSFAC (Transcriptional Factor Database). The data on the patterns of gene expression will be correlated with additional experimental information.

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APPENDIX A

Genes Up-regulated by Treatment with 17-β-estradiol in Wild Type A. thaliana

	Probe ID	Gene Description	Public ID	Fold-change
Transcription factors	246911_at	Transcription factor TINY	At5g25810	2.1209238
	258975_at	Putative WRKY-like transcriptional regulator protein similar to WRKY1 GB: AAC49527 (Petroselinum crispum); supported by full-length cDNA: Ceres: 1415.	At3g01970	2.238593
	253872_at	Putative protein Arabidopsis thaliana nap gene, PID: e1234813; supported by full-length cDNA: Ceres: 38344.	At4g27410	2.2875372
	266841_at	Putative heat shock transcription factor	At2g26150	2.1682266
Metabolism and Signaling	263320_at	Putative galactinol synthase; supported by full-length cDNA: Ceres: 124236.	At2g47180	2.0068289
	250268_s_at	Putative protein putative secreted protein SCF41.30c, Streptomyces coelicolor, EMBL:SCF41_30	At5g12950	2.0292998
	259875_s_at	12-oxophytodienoate reductase (OPR2) identical to 12-oxophytodienoate reductase OPR2 GB:AAC78441 [Arabidopsis thaliana]	At1g76690	2.034709
	267263_at	Similar to late embryogenesis abundant proteins; supported by cDNA: gi_14423503_gb_AF386989.1_AF386989	At2g23110	2.0349356
	248125_at	2S storage protein-like	At5g54740	2.8378202

Metabolism and Signaling	260408_at	Putative thioredoxin similar to thioredoxin GB:S58123 [Arabidopsis thaliana]	At1g69880	2.068955
	263137_at	Gamma glutamyl hydrolase, putative similar to gamma glutamyl hydrolase GI:1680711 from [Glycine max]; supported by cDNA: gi_16323195_gb_AY057702.1_	At1g78660	2.0711438
	249718_at	Putative protein contains similarity to beta-1, 3-glucanase; supported by full-length cDNA: Ceres: 11830.	At5g35740	2.0880535
	247983_at	Pyrophosphate-dependent phosphofructo-1-kinase-like protein; supported by cDNA:	At5g56630	2.1986251
	251428_at	Beta-glycosidase-like protein several beta-glucosidases - different species; supported by cDNA: gi_10834547_gb_AF159376.1_AF159376	At3g60140	2.2467327
	253268_s_at	Glucosyltransferase -like protein immediate-early salicylate-induced glucosyltransferase, Nicotiana tabacum, PIR2:T03747; supported by cDNA gi:14334981	At4g34135	2.2515002
	247283_at	2-nitropropane dioxygenase-like protein; supported by full-length cDNA: Ceres: 207555.	At5g64250	2.4005228
	266299_at	Glutathione S-transferase identical to GB: X89216; supported by full-length cDNA: Ceres: 6528.	At2g29450	2.4455176
	260803_at	Glutathione transferase, putative similar to glutathione transferase GI: 2853219 from [Carica papaya]; supported by full-length cDNA: Ceres: 252874	At1g78340	2.4991799

Metabolism and Signaling	262518_at	Putative glutathione transferase One of three repeated putative glutathione transferases. 72% identical to glutathione transferase [Arabidopsis thaliana] (gi|4006934)	At1g17170	2.8178293
	266270_at	Putative glutathione S-transferase; supported by cDNA: gi_11096003_gb_AF288185.1_AF288185	At2g29470	3.342769
	266267_at	Putative glutathione S-transferase; supported by cDNA: gi_14423533_gb_AF387004.1_AF387004	At2g29460	4.5897804
	258815_at	Putative short-chain type dehydrogenase/reductase similar to short-chain type dehydrogenase/reductase GB:Q08632 [Picea abies]; supported by cDNA: gi_15027900_gb_AY045807.1_	At3g04000	5.6515599
	253767_at	12S cruciferin seed storage protein	At4g28520	2.6257497
	261242_at	Subtilisin-like serine protease contains similarity to subtilase; SP1 GI:9957714 [Oryza sativa]	At1g32960	3.7628616
	256181_at	Light repressible receptor protein kinase, putative similar to light repressible receptor protein kinase GI:1321686 from (Arabidopsis thaliana)	At1g51820	2.1193428
	263948_at	Similar to harpin-induced protein hin1 from tobacco; supported by full-length cDNA: Ceres: 26418.	At2g35980	3.2987198
	248686_at	33 kDa secretory protein-like; supported by cDNA: gi_15292980_gb_AY050924.1_	At5g48540	2.0772013
	262930_at	Hypothetical protein similar to hin1 GB: Y07563 GI: 1619320	At1g65690	2.0972713

Metabolism and Signaling		Nicotiana tabacum; supported by full-length cDNA: Ceres: 21639.		
Flavonoid Metabolism	251971_at	Glucosyltransferase - like protein glucosyltransferase IS5a, Nicotiana tabacum, PIR:T03747	At3g53160	2.3757349
HSPs and Stress Response	253884_at	Heat shock protein 21	At4g27670	2.0614106
	258984_at	Putative DnaJ protein Pfam HMM hit: DnaJ, prokaryotic heat shock protein	At3g08970	2.0734702
	249575_at	Low-molecular-weight heat shock protein - like cytosolic class I small heat-shock protein HSP17.5, Castanea sativa, EMBL:CSA9880	At5g37670	2.1712713
	263150_at	Heat-shock protein, putative similar to heat-shock protein GI: 472939 from [Helianthus annuus]; supported by full-length cDNA: Ceres: 97415.	At1g54050	2.2097008
	261838_at	Heat shock protein hsp70, putative similar to heat shock protein hsp70 GI:1771478 from [Pisum sativum]	At1g16030	2.2309907
	262148_at	Chloroplast-localized small heat shock protein, putative similar to chloroplast-localized small heat shock protein GI:6601536 from [Funaria hygrometrica]	At1g52560	2.4425945
	262911_s_at	Heat shock protein, putative similar to heat shock protein GI: 19617 from [Medicago sativa]; supported by full-length cDNA: Ceres: 32795.	At1g59860	2.4793845
	260248_at	Heat shock protein 101 (HSP101) identical to heat shock protein 101 GI:6715468 GB:AAF26423 from [Arabidopsis thaliana]	At1g74310	2.4845595

HSPs and Stress Response	255811_at	Heat shock protein 22.0; supported by cDNA: gi_511795_gb_U11501.1_ATU11501	At4g10250	2.6632058
	252515_at	Heat shock protein 17; supported by cDNA: gi_15294149_gb_AF410266.1_AF410266	At3g46230	2.8001142
	250351_at	Heat shock protein 17.6A	At5g12030	2.996809
	260978_at	17.6 kDa heat shock protein (AA 1-156) identical to GI:4376161 from (Arabidopsis thaliana) (Nucleic Acids Res. 17 (19), 7995 (1989))	At1g53540	3.1069771
	250296_at	Heat shock protein 17.6-II; supported by full-length	At5g12020	3.2194472
	257264_at	Hypothetical protein contains Pfam profile: PF01657 Domain of unknown function; supported by cDNA: gi_14334417_gb_AY034900.1_	At3g22060	5.2440823
	258957_at	Feebly-like protein contains similarity to feebly protein GB:S70648 [Lycopersicon esculentum]; supported by cDNA: gi_14595998_gb_AY042787.1_	At3g01420	2.1028348
	249767_at	Acidic endochitinase (dbj|BAA21861.1)	At5g24090	2.7032142
	252102_at	Dehydrin Xero2; supported by cDNA: gi_15809983_gb_AY054260.1_	At3g50970	2.6503169
	254889_at	Osmotin precursor; supported by full-length cDNA: Ceres: 13796.	At4g11650	2.4878205
P450	258114_at	Putative cytochrome P450 similar to GB:Q05047 from [Catharanthus roseus]	At3g14660	2.0030832
	256589_at	Cytochrome P450, putative contains Pfam profile: PF00067 cytochrome P450; supported by cDNA: gi_15292830_gb_AY050849.1_	At3g28740	2.3181821

P450	266995_at	Putative cytochrome P450	At2g34500	2.3285647
Transporters	261448_at	Tonoplast intrinsic protein, alpha (alpha-TIP) similar to GB:X16488 from [Glycine max] (Plant Mol. Biol. 14 (3), 449-451 (1990))	At1g21140	2.025295
	267008_at	Putative ABC transporter	At2g39350	2.040027
	261763_at	ABC transporter, putative similar to ABC transporter GI:9279716 from [Arabidopsis thaliana]	At1g15520	3.5955043
	254723_at	Ammonium transport protein (AMT1); supported by cDNA: gi_14335079_gb_AY037219.1_	At4g13510	2.1045427
	258287_at	Putative sulfate transporter similar to sulfate transporter GB:BAA75015 from [Arabidopsis thaliana]; supported by cDNA: gi_12381948_dbj_AB054645.1_AB054645	At3g15990	2.1915127
	253829_at	Medicago nodulin N21-like protein MtN21 gene, Medicago truncatula, Y15293; supported by cDNA: gi_13899060_gb_AF370525.1_AF370525	At4g28040	2.1992805
	248551_at	Putative protein similar to unknown protein	At5g50200	2.3336138
	256548_at	Hypothetical protein contains similarity to MtN3(nodulin) protein GB:Y08726 GI:1619601 from [Medicago truncatula]; supported by cDNA: gi_15809922_gb_AY054229.1_	At3g14770	2.9057305
Horm.-related	250279_at	ABA-responsive protein - like ABA-responsive protein, Hordeum vulgare, EMBL:AF026538	At5g13200	2.2949698

Horm.-related	264912_at	Auxin-induced protein, putative similar to auxin-induced atb2 GI:6562980 from [Arabidopsis thaliana]	At1g60750	3.673437
Others	262682_at	Anter-specific proline-rich -like protein (APG-like) similar to anter-specific proline-rich protein (APG) SP:P40602 [Arabidopsis thaliana (Mouse-ear cress)]; supported by cDNA: gi_15054385_gb_AY028611.1_	At1g75900	3.9676947
Unknown	262349_at	Unknown protein; supported by full-length cDNA: Ceres: 32411	At2g48130	2.0016526
	258327_at	Unknown protein contains similarity to major storage protein GB:384341 from [Theobroma cacao]; supported by cDNA: gi_16604373_gb_AY058085.1_	At3g22640	2.0545253
	261265_at	Hypothetical protein predicted by genscan+; supported by full-length cDNA: Ceres: 250127.	At1g26800	2.2084559
	254823_at	Putative protein predicted protein	At4g12580	2.2105788
	251443_at	Putative protein unknown protein At2g44130 - Arabidopsis thaliana, EMBL: AC004005; supported by full-length cDNA: Ceres: 8014.	At3g59940	2.3400757
	260881_at	Unknown protein contains similarity to calcium-binding protein GB:CAB63264 GI:6580549 from [Lotus japonicus]; supported by cDNA: gi_13605536_gb_AF361594.1_AF361594	At1g21550	2.3609796
	262503_at	Hypothetical protein predicted by genscan; supported by cDNA: gi_14334811_gb_AY035079.1_	At1g21670	2.463089

Unknown	257226_at	Hypothetical protein predicted by genemark.hmm; supported by full-length cDNA: Ceres: 15282.	At3g27880	2.4780721
	252170_at	Hypothetical protein; supported by cDNA: gi_13605735_gb_AF361849.1_AF361849	At3g50480	3.1433026
	266486_at	Hypothetical protein predicted by genscan and genefinder; supported by full-length cDNA: Ceres: 31153.	At2g47950	3.4202034
	266364_at	Unknown protein	At2g41230	2.8634845
	267238_at	Unknown protein; supported by full-length cDNA: Ceres: 6950.	At2g44130	8.217452
	262229_at	Unknown protein; supported by cDNA: gi_14335125_gb_AY037242.1_	At1g68620	2.4920839

APPENDIX B

Genes down-regulated by treatment with 17-β-estradiol in wild type A. thaliana

	Probe ID	Gene Description	Public ID	Fold-change
Transcription factors	256647_at	Unknown protein contains similarity to DNA-binding protein zyxin GB:X99063 GI:1430882 from [Mus musculus]	At3g13610	0.3936418
	247549_at	Putative transcription factor MYB28; supported by cDNA: gi_5823328_gb_AF175998.1_AF175998	At5g61420	0.40426747
	266455_at	Putative bHLH transcription factor	At2g22760	0.45813446
	254231_at	Putative protein AR411 - Arabidopsis thaliana (thale cress), PID:g1669603; supported by cDNA: gi_13507100_gb_AF272748.1_AF272748	At4g23810	0.47053221
	263584_at	NAM (no apical meristem)-like protein similar to petunia NAM (X92205) and A. thaliana sequences ATAF1 (X74755) and ATAF2 (X74756); probable DNA-binding protein; supported by cDNA: gi_13605646_gb_AF361804.1_AF361804	At2g17040	0.47004471
	245628_at	Anthocyanin2, putative similar to anthocyanin2 (An2) GI:7673088 from [Petunia integrifolia]; supported by cDNA: gi_3941507_gb_AF062908.1_AF062908	At1g56650	0.41987114
	246522_at	BZIP DNA-binding protein-like putative bZIP DNA-binding protein - Capsicum chinense, EMBL:AF127797	At5g15830	0.47773567

Transcription factors	254074_at	Transcriptional activator CBF1 CRT CRE binding factor 1 involved in low-temperature-responsive gene expression00; supported by cDNA: gi_1899057_gb_U77378.1_ATU77378	At4g25490	0.450854
	249890_at	Putative protein contains similarity to DNA-binding protein	At5g22570	0.49851581
	253070_at	Putative protein symbiotic ammonium transporter SAT1, Glycine max, AF069738	At4g37850	0.3300829
	260451_at	Putative AP2 domain transcription factor contains Pfam	At1g72360	0.45672936
Metabolism and Signaling	259388_at	Steroid sulfotransferase, putative similar to steroid sulfotransferase 1 GI: 3420004 from (Brassica napus); supported by full-length cDNA: Ceres: 150484.	At1g13420	0.25184233
	258473_s_at	Putative stearoyl-acyl carrier protein desaturase similar to stearoyl-acyl carrier protein desaturase GB:CAA07349 from [Linum usitatissimum]	At3g02610	0.32418355
	248729_at	Cycloartenol synthase	At5g48010	0.47578
	256012_at	Unknown protein similar to dimethylaniline monooxygenase (N-oxide-forming)-like protein GI:9759603 from [Arabidopsis thaliana]	At1g19250	0.25525747
	249188_at	N-hydroxycinnamoyl benzoyltransferase-like protein	At5g42830	0.28245876
	253697_at	Nucleotide pyrophosphatase -like protein nucleotide pyrophosphatase, Oryza sativa, gb:T03293; supported by cDNA: gi_13430713_gb_AF360269.1_AF360269	At4g29700	0.3077508
	257021_at	Branched-chain amino acid aminotransferase, putative similar to branched-chain acid aminotransferase GB:AAF07192 from Solanum	At3g19710	0.3199716

Metabolism and Signaling		tuberosum		
	251524_at	3-isopropylmalate dehydratase-like protein (small subunit) 3-isopropylmalate dehydratase, small subunit - Thermotoga maritima, PIR:A72363	At3g58990	0.32297477
	260093_at	Putative serine carboxypeptidase similar to serine carboxypeptidase I precursor GB:P07519 [Hordeum vulgare], glucose acetyltransferase GB:AAD01263 [Solanum berthaultii]; contains Pfam profile: PF00450 Serine carboxypeptidase	At1g73270	0.48142656
	260547_at	Putative trypsin inhibitor; supported by full-length cDNA	At2g43550	0.48225303
	250083_at	Glutathione S-transferase-like protein; supported by cDNA: gi_11096011_gb_AF288189.1_AF288189	At5g17220	0.32659791
	259813_at	Glutathione S-transferase, putative similar to GI:860955 from [Hyoscyamus muticus] (Plant Physiol. 109 (1), 253-260 (1995))	At1g49860	0.38786016
	247577_at	Putative protein flavin-containing monooxygenase FMO3, Oryctolagus cuniculus, SWISSPROT:FMO3_RABIT; supported by full-length	At5g61290	0.34821443
	245258_at	Lupeol synthase like protein; supported by cDNA: gi_6650207_gb_AF062513.1_AF062513	At4g15340	0.35569187
	245624_at	Glucosyltransferase like protein	At4g14090	0.35779327
	249866_at	2-isopropylmalate synthase-like; homocitrate synthase-like; supported by cDNA: gi_12330688_gb_AF327648.1_AF327648	At5g23010	0.37081987
	248625_at	3-keto-acyl-CoA thiolase 2 (gb|AAC17877.1); supported by cDNA:	At5g48880	0.37553311

Metabolism and Signaling		gi_3192892_gb_AF062590.1_AF062590		
	246149_at	Peroxidase ATP N; supported by full-length cDNA: Ceres: 40493.	At5g19890	0.39939138
	256601_s_at	At14a-1 protein identical to At14a protein GB:AAD26355 GI:4589123 [Arabidopsis thaliana]	At3g28290	0.38914533
	258322_at	Putative selenocysteine methyltransferase similar to selenocysteine methyltransferase GB: P56707 from [Astragalus bisulcatus]; supported by full-length cDNA: Ceres: 36591.	At3g22740	0.41305659
	262619_at	3-hydroxyisobutyryl-coenzyme A hydrolase, putative similar to 3-hydroxyisobutyryl-coenzyme A hydrolase GB:AAC52114 GI:3320120 [Homo sapiens]	At1g06550	0.4934078
	254914_at	Peroxidase ATP19a	At4g11290	0.49417594
	247867_at	SNF1 related protein kinase-like protein; supported by cDNA: gi_14334389_gb_AY034100.1_	At5g57630	0.49698985
	245736_at	Dr4 (protease inhibitor) identical to Dr4 GI:469114 from [Arabidopsis thaliana]; supported by cDNA: gi_13877842_gb_AF370184.1_AF370184	At1g73330	0.421041
	249600_s_at	Oxidoreductase-like protein zeta-crystallin homologue, putative NADPH oxidoreductase - Arabidopsis thaliana, EMBL:Z49768	At5g38000	0.42429308
	267128_at	Putative acetone-cyanohydrin lyase	At2g23620	0.42701803
	259264_at	Putative aldose 1-epimerase shows similarity to aldose epimerases	At3g01260	0.43154267
	263451_at	Putative triacylglycerol lipase	At2g31690	0.43397168

Metabolism and Signaling	245928_s_at	Vegetative storage protein Vsp1; supported by full-length cDNA: Ceres: 32606.	At5g24780	0.43511243
	258103_at	Hypothetical protein contains Pfam profile: PF01715 IPP transferase; supported by full-length cDNA: Ceres: 40185.	At3g23630	0.43655516
	260153_at	Putative lipase similar to monoglyceride lipase GB: NP_035974 from [Mus musculus]; supported by full-length cDNA: Ceres: 36954.	At1g52760	0.4392276
	249867_at	2-isopropylmalate synthase-like protein	At5g23020	0.44091425
	251195_at	Glutaredoxin -like protein glutaredoxin, castor bean, PIR:S54825	At3g62930	0.44131534
	258589_at	Putative GDSL-motif lipase/acylhydrolase contains Pfam profile: lipase/acylhydrolase with GDSL-like motif; supported by full-length cDNA: Ceres: 1323.	At3g04290	0.44360313
	261913_at	Flavin-containing monooxygenase FMO3, putative similar to flavin-containing monooxygenase FMO3 GI:349533 from [Oryctolagus cuniculus]	At1g65860	0.44828042
	262646_at	Aspartate aminotransferase nearly identical to aspartate aminotransferase, cytoplasmic isozyme 2 SP:P46646 [Arabidopsis thaliana (Mouse-ear cress)]; supported by cDNA: gi_693693_gb_U15035.1_ATU15035	At1g62800	0.44899626
	260130_s_at	Beta-glucosidase, putative similar to GB:AAB64244 from [Arabidopsis thaliana] (Plant Mol. Biol. 34 (1), 57-68 (1997))	At1g66280	0.45038124
	254737_at	Fatty acid elongase-like protein (cer2-like) cer2, Arabidopsis thaliana,	At4g13840	0.46545483

Metabolism and Signaling		X93080		
	265121_at	Similar to flavin-containing monooxygenase (sp|P36366); similar to ESTs gb|R30018, gb|H36886, gb|N37822, and gb|T88100 similar to flavin-containing monooxygenase GB:AAA21178 GI:349534 from [Oryctolagus cuniculus]; supported by cDNA: gi_13877746_gb_AF370136.1_AF370136	At1g62560	0.46726238
	259951_at	Glutathione S-transferase identical to glutathione S-transferase GB:AAB09584 from Arabidopsis thaliana	At3g19710	031398523
	264527_at	Putative reticuline oxidase-like protein similar to GB:P30986 from [Eschscholzia californica] (berberine bridge-forming enzyme), ESTs gb|F19886, gb|Z30784 and gb|Z30785 come from this gene	At1g30760	0.47558601
	263594_at	Putative purple acid phosphatase contains metallo-phosphoestarase motif (PS50185)	At2g01880	0.48374111
	261991_at	Hypothetical protein predicted by genemark.hmm	At1g33700	0.42887098
	245555_at	HSR201 like protein	At4g15390	0.43698622
Flavonoid Metabolism	256368_at	Cinnamyl alcohol dehydrogenase, putative similar to cinnamyl alcohol dehydrogenase [Eucalyptus gunnii] GI:1143445	At1g66800	0.26818517
	258023_at	Cinnamyl alcohol dehydrogenase identical to GB: P48523 from [Arabidopsis thaliana]; supported by full-length cDNA: Ceres: 4357.	At3g19450	0.4493361
	249215_at	Dihydroflavonol 4-reductase	At5g42800	0.28290285
	253088_at	Ferulate-5-hydroxylase (FAH1); supported by cDNA:	At4g36220	0.29634528

Flavonoid Metabolism		gi_1488254_gb_U38416.1_ATU38416		
	265091_s_at	Hypothetical protein similar to Anthocyanin 5-aromatic acyltransferase GB:BAA74428	At1g03495	0.30837114
	258037_at	Putative 4-coumarate:CoA ligase 2 similar to GB:AAD47192 from [Arabidopsis thaliana]	At3g21230	0.32332665
	256186_at	4-coumarate:CoA ligase 1 identical to 4-coumarate:CoA ligase 1 [Arabidopsis thaliana] GI:5702184; supported by cDNA: gi_609339_gb_U18675.1_ATU18675	At1g51680	0.48226567
	262325_at	Dirigent protein, putative similar to dirigent protein GB:AAF25365 GI:6694709 from [Thuja plicata]	At1g64160	0.33724945
	248185_at	Flavonol 3-O-glucosyltransferase-like	At5g54060	0.3427757
	255773_at	Flavonol 4'-sulfotransferase, putative similar to flavonol 4'-sulfotransferase GI:168168 from [Flaveria chloraefolia]	At1g18590	0.45376337
	260385_at	Putative flavonol sulfotransferase similar to FLAVONOL 4'-SULFOTRANSFERASE GB: P52837 from [Flaveria chloraefolia]; supported by full-length cDNA: Ceres: 41006.	At1g74090	0.48854149
	266828_at	Putative flavonol 3-O-glucosyltransferase	At2g22930	0.46855514
	250558_at	Flavonoid 3-hydroxylase - like protein flavonoid 3'-hydroxylase Ht1, Petunia x hybrida, EMBL:AF155332; supported by cDNA: gi_10334803_gb_AF271649.1_AF271649	At5g07990	0.44783561
	258047_at	Putative 4-coumarate:CoA ligase 2 almost identical (1 amino acid	At3g21240	0.34684205

Flavonoid Metabolism		difference) to GB:AAD47192 from [Arabidopsis thaliana]; supported by cDNA: gi_5702187_gb_AF106086.1_AF106086		
	259149_at	Putative phenylalanine ammonia-lyase similar to phenylalanine ammonia-lyase GB:S48726 [Petroselinum crispum]	At3g10340	0.37628862
	263845_at	Phenylalanine ammonia lyase (PAL1); supported by cDNA: gi_15028192_gb_AY045919.1_	At2g37040	0.42433389
	248209_at	Flavonol 3-O-glucosyltransferase-like protein	At5g53990	0.39051936
	261804_at	UDP glucose: flavonoid 3-o-glucosyltransferase, putative similar to UDP glucose: flavonoid 3-o-glucosyltransferase GB: AAB81683 GI: 2564114 from [Vitis vinifera]; supported by full-length cDNA: Ceres: 38407.	At1g30530	0.431025
	256924_at	Anthocyanin 5-aromatic acyltransferase, putative similar to Anthocyanin 5-aromatic acyltransferase GB:BAA74428 from [Gentiana triflora]	At3g29590	0.41434524
	254283_s_at	Anthocyanidin synthase - like protein putative leucoanthocyanidin dioxygenase, Arabidopsis thaliana, PID:g1575699	At4g22870	0.43081461
	261907_at	4-coumarate:CoA ligase 3 identical to 4-coumarate:CoA ligase 3 GI:5702190 from [Arabidopsis thaliana]; supported by cDNA: gi_5702191_gb_AF106088.1_AF106088	At1g65060	0.42438085
	250794_at	Putative protein contains similarity to chalcone-flavonone isomerase (chalcone isomerase) supported by full-length cDNA: Ceres: 40439.	At5g05270	0.36964518
	251827_at	Chalcone isomerase; supported by full-length cDNA: Ceres: 2122.	At3g55120	0.37030516

Flavonoid Metabolism				
HSPs and Stress Response	254907_at	Putative disease resistance response protein disease resistance response protein 206-d - Pisum sativum, PID:g508844	At4g11190	0.4708498
	254092_at	Respiratory burst oxidase - like protein respiratory burst	At4g25090	0.38943587
	258618_at	Expressed protein; supported by cDNA: gi_1289319_gb_U53221.1_ATU53221	At3g02885	0.4905133
	247215_at	Expressed protein; supported by full-length cDNA: Ceres: 3657.	At5g64905	0.4487082
	254440_at	Putative protein desiccation-related protein, Craterostigma plantagineum, PIR2:C45509	At4g21020	0.47927337
P450	266218_s_at	Putative cytochrome P450	At2g28850	0.18656844
	266996_at	Putative cytochrome P450; supported by full-length cDNA: Ceres: 158108.	At2g34490	0.38139878
	249202_at	Cytochrome P450	At5g42580	0.29418236
	249203_at	Cytochrome P450	At5g42590	0.41472259
	248727_at	Cytochrome P450	At5g47990	0.47743058
	245550_at	Cytochrome P450-like protein	At4g15330	0.49045679
	248728_at	Cytochrome P450-like protein	At5g48000	0.41982747
	254687_at	Cytochrome P450 monooxygenase (CYP83A1); supported by cDNA: gi_3164127_dbj_D78599.1_D78599	At4g13770	0.34531585
	256801_at	Cytochrome P450, putative similar to cytochrome P450 GB:H71417	At3g20940	0.41336079

P450		from [Arabidopsis thaliana]		
	262717_s_at	Putative cytochrome P450 similar to gb|AF069494 cytochrome P450 from Sinapis alba and is a member of the PF|00067 Cytochrome P450 family. EST gb|F14190 comes from this gene; supported by cDNA: gi_15208670_gb_AY035021.2_	At1g16410	0.29904202
Transporters	246238_at	Sugar transporter like protein	At4g36670	0.38563739
	254862_at	Putative transport protein Na(+) dependent transporter (Sbf family) - Aquifex aeolicus, PIR2:E70482; supported by cDNA: gi_15215838_gb_AY050449.1_	At4g12030	0.36609603
	252537_at	Putative transporter protein peptide transport protein - Hordeum vulgare, PIR:T04378	At3g45710	0.39842209
	252536_at	Putative transporter protein peptide transport protein - Hordeum vulgare, PIR:T04378	At3g45700	0.46208136
Hormone Related	249675_at	Putative protein myrosinase-binding protein-like; also similar to jasmonate inducible protein-like	At5g35940	0.46186461
Others	253309_at	Male sterility 2-like protein male sterility protein 2, Brassica napus, gb:X99922; supported by cDNA: gi_16323106_gb_AY057657.1_	At4g33790	0.38082771
	262838_at	Major latex protein, putative similar to major latex-like protein GB: CAA11844 GI: 3164115 [Rubus idaeus]; supported by full-length cDNA: Ceres: 3858.	At1g14960	0.45714707
Unknown	247205_at	Unknown protein; supported by full-length cDNA: Ceres: 9242.	At5g64890	0.33581966

	247213_at	Unknown protein; supported by full-length cDNA: Ceres: 25655.	At5g64900	0.45403787
	249814_at	Putative protein similar to unknown protein (pir||T00970); supported by full-length cDNA: Ceres: 4716.	At5g23840	0.45969383
	252944_at	Hypothetical protein; supported by full-length cDNA: Ceres: 14629.	At4g39320	0.46810222
	247582_at	Putative protein various predicted proteins, Arabidopsis thaliana	At5g60760	0.47912908
	260883_at	Hypothetical protein predicted by genemark.hmm	At1g29270	0.47926048
	263829_at	Unknown protein	At2g40435	0.48334158
	262237_at	Hypothetical protein predicted by genemark.hmm	At1g48320	0.48802273
	260178_at	Hypothetical protein similar to unknown protein GB: AAC00599 [Arabidopsis thaliana]; supported by full-length cDNA: Ceres: 21812.	At1g70720	0.48913671
	266975_at	Hypothetical protein predicted by grail	At2g39380	0.49726721

APPENDIX C

Non-responsive wild type Arabidopsis genes to 17-β-estradiol treatment

Probe ID	Gene Description	Public ID	Fold-change
265672_at	Putative cysteine proteinase inhibitor B (cystatin B); supported by full-length cDNA: Ceres: 35447.	At2g31980	0.55763
259432_at	Myb-related protein, putative similar to myb-related protein GI:2505876 from [Arabidopsis thaliana]	At1g01520	0.589193
258498_at	Unknown protein similar to pollen coat protein GB:CAA63531 from [Brassica oleracea]; supported by cDNA: gi_14335127_gb_AY037243.1_	At3g02480	0.615746
264494_at	Hypothetical protein predicted by genefinder	At1g27470	0.634888
258347_at	Unknown protein identical to LEA-like protein GB:CAA10352 from [Arabidopsis thaliana]	At3g17520	0.650524
247061_at	Putative protein similar to unknown protein (pir |T09249); supported by cDNA: gi_15081693_gb_AY048239.1_	At5g66780	0.657804
262347_at	Unknown protein similar to hypothetical protein GB:AAF24564 GI:6692099 from [Arabidopsis thaliana]; supported by cDNA: gi_15810166_gb_AY056097.1_	At1g64110	0.771268
265211_at	Late embryogenesis abundant protein (AtECP63) ; supported by cDNA: gi_1526423_dbj_D64140.1_D64140	At2g36640	0.77178
250648_at	Late embryogenesis abundant protein LEA like ; supported by cDNA: gi_15293004_gb_AY050936.1_	At5g06760	0.777254
256464_at	Late-embryogenesis abundant protein, putative similar to GI:4102692 from [Glycine max]	At1g32560	0.792775

259167_at	Putative oleosin similar to oleosin GB:AAB58402 [Sesamum indicum]; supported by cDNA: gi_16649132_gb_AY059936.1_	At3g01570	0.847099
263138_at	Hypothetical protein predicted by genemark.hmm	At1g65090	0.869355
249039_at	Late embryogenesis abundant protein-like	At5g44310	0.876455
261648_at	Salt-tolerance zinc finger protein identical to salt-tolerance zinc finger protein GB:CAA64820 GI:1565227 from [Arabidopsis thaliana]; supported by cDNA: gi_14334649_gb_AY034998.1_	At1g27730	0.93366
267321_at	Hypothetical protein predicted by genefinder	At2g19320	0.933812
256338_at	Hypothetical protein similar to seed maturation protein PM27 [Glycine max] GI:4836403	At1g72100	0.94264
262069_at	Hypothetical protein similar to SNF4-kinase activating protein GI:7672780 from [Lycopersicon esculentum]	At1g80090	0.952355
264612_at	Unknown protein similar to GB:AAC37469; supported by cDNA: gi_15293292_gb_AY051080.1_	At1g04560	1.022096
254634_at	Putative protein transcription factor OBF3.1, Zea mays,	At4g18650	1.041669
245335_at	Pore protein homolog; supported by full-length cDNA: Ceres: 5714.	At4g16160	1.068448
256827_at	Hypothetical protein contains similarity to Pfam profile:	At3g18570	1.080273
266274_at	Putative protein phosphatase 2C	At2g29380	1.109969
252137_at	Dehydrin-like protein dehydrin Xero2 - Arabidopsis thaliana, EMBL:U19536	At3g50980	1.116687
259217_at	Unknown protein similar to F16N3.20 GB:AAD46034 [Arabidopsis thaliana], unknown protein GB:CAA66809 [Arabidopsis thaliana]	At3g03620	1.121367

263753_at	Putative dehydrin; supported by full-length cDNA: Ceres: 5256.	At2g21490	1.136688
262185_at	MADS box transcription factor, putative similar to MADS box transcription factor GI:1905943 from [Sorghum bicolor]	At1g77950	1.217545
251838_at	Cysteine proteinase non-consensus AG donor site at exon 2; contains similarity to cysteine proteinase GI:479060 from [Glycine max]	At3g54940	1.240352
257994_at	Unknown protein	At3g19920	1.474821