Journal of
Experimental
Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Transcriptome profiling of cytokinin and auxin regulation in tomato root

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Abstract

Tomato is a model and economically important crop plant with little information available about gene expression in roots. Currently, there have only been a few studies that examine hormonal responses in tomato roots and none at a genome-wide level. This study examined the transcriptome atlas of tomato root regions (root tip, lateral roots, and whole roots) and the transcriptional regulation of each root region in response to the plant hormones cytokinin and auxin using Illumina RNA sequencing. More than 165 million 1 × 54 base pair reads were mapped onto the *Solanum lycopersicum* reference genome and differential expression patterns in each root region in response to each hormone were assessed. Many novel cytokinin- and auxin-induced and -repressed genes were identified as significantly differentially expressed and the expression levels of several were confirmed by qPCR. A number of these regulated genes represent tomato orthologues of cytokinin- or auxin-regulated genes identified in other species, including *CKX*s, type-A *RR*s, *Aux/IAA*s, and *ARF*s. Additionally, the data confirm some of the hormone regulation studies for recently examined genes in tomato such as Sl*IAA*s and Sl*GH3*s. Moreover, genes expressed abundantly in each root region were identified which provide a spatial distribution of many classes of genes, including plant defence, secondary metabolite production, and general metabolism across the root. Overall this study presents the first global expression patterns of hormone-regulated transcripts in tomato roots, which will be functionally relevant for future studies directed towards tomato root growth and development.

Key words: Auxin, cytokinin, lateral root, RNA sequencing, root, root tip, tomato.

Introduction

Tomato (*Solanum lycopersicum*) is one of the main crops grown throughout the world. Many studies toward the improvement and increase in yield of this plant have focused on flower (Goméz *et al.*, 1999; Molinero-Rosales *et al.*, 2003) and fruit development (de Freitas *et al.*, 2012; Pattison and Catalá, 2012). Roots in tomato, on the other hand, which constitute an important system to carry out water conduction, mineral uptake, anchorage, synthesis of bio-active substances, gravitropic responses, and sensing stress have not been studied in detail. This is particularly true for gene expression patterns in tomato roots that will be essential for

any future studies focused on growing, breeding, or engineering plants with more desirable root traits.

The root system comprises different regions that are involved in specific functions for the overall plant sustenance. Two essential regions are the root tips and lateral roots. The root tip encompasses the root apical meristem, which provides all the cells and base organization for the growing root (Scheres *et al.*, 2002). Moreover, this is a region of active cell division, elongation (Dolan *et al.*, 1993), and is also the region where synthesis of important hormones (Feldman, 1979; Muller *et al.*, 1998) occurs which are transported to different

locations in the plant. Lateral roots on the other hand extend from the primary root in horizontal manner to provide support and increased water and nutrient uptake.

Two key hormones that play pivotal roles in root growth and development are cytokinin and auxin. Both of these hormones have been implicated to have roles in several processes such as in root vascular development, initiation of lateral roots, and root gravitropism (Aloni *et al.*, 2006; Vanneste and Friml, 2009). Quite often, cytokinin displays crosstalk with auxin during root development, such as in the establishment of root stem-cell niche (Müller and Sheen, 2008), meristem size and root growth (Dello Ioio *et al.*, 2008; Zhang *et al.*, 2011), and lateral root organogenesis (Marhavý *et al.*, 2011), although sometimes in an antagonistic manner. Through several studies, the regulatory roles of hormones in plant development have been elucidated. This includes several cytokinin-induced genes, such as the *CRE1/AHK4* cytokinin receptor (Che *et al.*, 2002), *CRF*s that are involved in *Arabidopsis* cotyledon and leaf development (Rashotte *et al.*, 2006), and a *O*-glucosyltransferase in rice that catalyses the inactivation of zeatin-type CKs by *O*-glucosylation (Hirose *et al.*, 2007). Numerous auxin-regulated genes have similar important roles, such as *SAURs* that are strongly expressed in epidermal and cortical cells (Vanneste and Friml, 2009) and *AUF1* that is involved in crosstalk between cytokinin and auxin during *Arabidopsis* root growth (Zheng *et al.*, 2011).

With the availability of the complete tomato genome (The Tomato Genome Consortium, 2012), it has become possible to perform genome-wide transcriptome analysis to study gene expression patterns across different plant tissues under different conditions without *de novo* assembly. Next-generation high-throughput RNA sequencing technology (RNA-seq) using massively parallel sequencing has revolutionized transcriptome analysis and, when compared to microarrays, RNA-seq can detect all expressed genes without the generation of an array of probes, with reduced background noise and large dynamic range. This is particularly important in species such as tomato, where publicly available microarrays cover only one-third of the complete genome.

This study utilized Illumina RNA sequencing on 15-day-old hydroponically grown Micro-Tom plants to analyse the transcriptome of tomato roots with the main focus on the spatial patterning and regulation of genes in the root by the hormones cytokinin and auxin. This study is one of only a few tomato genome-wide expression profiles and generates a transcriptome atlas of tomato root regions: root tip, lateral roots, and whole roots. Additionally, this transcriptome analysis of hormone regulation in tomato root reveals novel genes regulated by each of these hormones. This comprehensive analysis of tomato root transcriptome can further be utilized as a reference to conduct future research on tomato roots.

Materials and methods

Plant materials, growth conditions, and hormone treatment

The tomato dwarf cultivar Micro-Tom plants were grown hydroponically in CYG germination pouches from Mega International under 26/22 °C, 16/8 h light/dark photoperiod at 150 µE. Seedlings at 14 days after sowing were treated with exogenous 5 µM cytokinin (N[6]-benzyladenine, BA) or 5 µM auxin (naphthalene acetic acid, NAA) dissolved in dimethylsulphoxide (DMSO) for 24 h by directly adding the hormones or control DMSO to the growth pouches, such that 15-day-old plant tissues were collected.

RNA extraction, library preparation, and Illumina GAIIX sequencing

Root tips (RT, encompassing the meristem and the elongation zone), lateral roots (LR), and whole roots (WR, including RT and LR) were collected from 15-day-old Micro-Tom plants from seedlings with or without treatment with cytokinin, auxin, and DMSO and immediately flash-frozen in liquid nitrogen and ground to obtain fine powder. Total RNA was extracted from the tissue using a Qiagen RNeasy Kit as per the manufacturer's instructions and used for messenger RNA isolation with polyA selection and subsequent library construction with the TruSeq RNA sample preparation protocol from Illumina (San Diego, CA). Two biological replicates were sequenced and analysed for each of the nine tissue–treatment combinations. Single-end sequencing was performed on the 18 samples by the Illumina GAIIX platform, generating 165,894,496 1×54 bp reads, totalling 8.99 Gbp. Raw sequence data is available for download at NCBI Sequence Read Archive under the number SRA058709.

Differential expression analysis with S. lycopersicum (ITAG2.3) reference

Reads for each of the 18 samples were aligned to the *S. lycopersicum* genome reference with GSNAP (Wu and Nacu, 2010). The high throughput sequencing data was managed using the Alpheus pipeline and database resource (Miller, 2008). Gene expression was quantified as the total number of reads for each sample that uniquely aligned to the reference, binned by gene coordinate. On average, each sample had 7.6 million uniquely aligning reads.

Differential expression analysis was performed with the negative binomial test of DESeq (Anders, 2010) on three partitions of the dataset: three tissues DMSO-only treatment, three tissues DMSO versus cytokinin treatment, and three tissues DMSO versus auxin treatment. To perform robust analyses, each set was normalized, filtered, and tested within DESeq separately with recommended settings. The first experiment interrogated tissue-specific signals with the DMSO-treated samples from root tip, lateral root, and whole root ($n = 6$). The second experiment investigated the effect of cytokinin treatment in each root tissue in reference to the corresponding DMSO control ($n = 12$). The final experiment looked at the effect of auxin treatment in each root tissue in reference to the corresponding DMSO control ($n = 12$). A multiple testing correction (the Benjamini–Hochberg false discovery rate method; Benjamini and Hochberg, 1995) was applied and genes with an adjusted *P*-value (P_{adj}) ≤0.05 were considered to be significantly differentially expressed. For multiple tissue differential expression, this study shows comparisons with a $\log_2$ fold-change that were significant ($P_{adj} \le 0.05$) in one or more of the root regions, indicating P_{adj} in all regions.

Verification of mRNA-seq results by quantitative real-time PCR

A 500 ng aliquot of the total RNA for each tissue type (see above) was used for reverse transcription using Quanta qScript cDNA supermix and the cDNA was diluted 20 times before it was used for analysis by quantitative real-time PCR (qPCR). Each reaction consisted of 2 µl 20-fold cDNA, 9 µl SYBR-Green supermix, 1 µl of 6 µM forward and reverse primers, and 7 µl sterile water. qPCR was performed in a Eppendorf Mastercycler ep realplex with the following parameters: one cycle of 2 min at 95 °C, followed by 40 cycles of

15 s at 95 °C, 45 s at 57 °C, and 35 s at 68 °C. The last step for each reaction was melting curve generation to test the amplicon specificity. All qPCR reactions were performed in two technical and two biological replicates. All samples were compared with the control gene *TIP41* (Expósito-Rodríguez *et al.*, 2008). The primer sequences for the genes which were verified through qPCR are presented in Supplementary Table S1 (available at *JXB* online).

Results and discussion

Tomato root transcriptome analysis

To obtain a global view of genes expressed across the tomato root, single-end Illumina GAIIX RNA sequencing was performed on samples derived from RT (encompassing the meristem and elongation zone), LR, and WR (including RT and LR) after 24 h of treatment with cytokinin (5 µM BA) or auxin (5 µM NAA) versus the vehicle control DMSO. This generated a total of 165,894,496 1×54 bp reads from all root tissue samples that were aligned to the *S. lycopersicum* reference genome. On average, samples generated 7.6 million uniquely aligning reads from which gene expression was quantified as the total number of reads for each sample that uniquely aligned to the reference, binned by gene.

Based on reads and filtering, ~17,300 genes were robust enough to be used for differential expression analysis on three subsets of the data – tissue-level expression patterns by utilizing untreated (DMSO) RT, LR, and WR samples – and the hormone expression in these tissues of cytokinin as well as auxin treatment in reference to DMSO. Since a large number of genes were found to be significantly regulated ($P_{adj} \leq 0.05$), study focused the differential expression analysis on genes with >2.0 log$_2$ fold-change, which were designated as hormone or tissue regulated (Table 1). The discussion of these results is concentrated on genes regulated commonly across the different tissues examined, as they represent some of the most highly regulated hormone-responsive genes with important known gene function in each root region. Furthermore, qPCR was performed in order to confirm the results of RNA sequencing on 12 differential expression-regulated genes affected by cytokinin and auxin treatments. This comparison yielded very similar expression directionality and level of regulation for all genes examined across all root tissues, indicating that the log$_2$ fold-change values obtained from RNA sequencing were accurate (Table 2).

Principal component analysis and variance decomposition (both as implemented in SAS JMP Genomics 5.1) of the overall, full transcriptome dataset ($n = 18$) showed distinct differences between the auxin and cytokinin treatments, responsible for 68.6% of the variance (Supplementary Fig. S1). With regards to tissue differences, 19.5% of the variance, there was greater similarity between LR and WR samples, while the RT has a more distinct profile.

Cytokinin-induced genes

Genes were determined to be cytokinin regulated if they had >2.0 log$_2$ fold-change in at least one root region sampled. This study identified 89 genes that were cytokinin regulated in all root tissues, while 87, 24, and 34 were specifically regulated in RT, LR, and WR tissues, respectively. Additionally, there were 24 genes commonly regulated in RT and LR tissues, 23 between LR and WR tissues, and 81 between RT and WR tissues (Fig. 1 and Supplementary Table S2).

Differentially expressed cytokinin-induced genes found in this study appear to be mainly involved in metabolism, development, defence, transport, and tissue-specific related processes. Some of the commonly found highly cytokinin-regulated genes are the cytokinin oxidase/dehydrogenases (*CKX*) (Brenner *et al.*, 2012). Three *CKX* genes (Sl*CKX2*, Sl*CKX4*, Sl*CKX6*) were found to be induced in all root tissues (Table 1), while Sl*CKX1* was induced specifically between RT and LR and Sl*CKX5* between RT and WR (Supplementary Table S1). *CKX* genes across plant genera are involved in the breakdown and catabolism of cytokinin (Frébort *et al.*, 2011), so it is not surprising to see several *CKX*s induced with the application of exogenous cytokinin. *CKX*s have also been linked to a number of cytokinin-related growth and developmental processes and recently it was shown that overexpression of *CKX* genes enhanced root growth, drought tolerance, and leaf mineral enrichment in *Arabidopsis* and tobacco (Werner *et al.*, 2010). Since this study has shown that *CKX*s can be highly induced in roots, it would be interesting to see if similar growth and developmental effects could be seen from *CKX* overexpression in tomato.

Seven type-A response regulator (RR) genes were found induced in this study, which are designated Sl*RRA*s. Sl*RRA2*, Sl*RRA6*, and Sl*RRA7* were induced in all root tissues (Table 1), Sl*RRA4* specifically in LR, and Sl*RRA1*, Sl*RRA3*, and Sl*RRA5* were induced between LR and WR (Supplementary Table S2). Type-A RRs are commonly found to be highly induced by cytokinin treatment as they are negative regulators of cytokinin signalling (Brenner *et al.*, 2012). It can be proposed that induction of these genes probably occurred to dampen the increased exogenous cytokinin levels added to the roots. Since cytokinin is a negative regulator of root growth, it would be interesting to know how the levels of cytokinin are maintained to appropriate amounts by the genes involved in cytokinin catabolism and the cytokinin signalling pathway components.

Defence genes, including those that produce volatile compounds in response to pathogens or predators, were among those induced by cytokinin, including the sesquiterpene synthase gene *TPS14* and (E)-beta-ocimene synthase (Solyc01g105960) (Table 1; Paré and Tumlinson, 1999). Sesquiterpenes such as alpha-humulene are widely distributed in plants and have been shown to function as insect/pathogen repellent in direct defence (Suga *et al.*, 1993). In support of the present results, *TPS14* has been shown to be highly expressed in tomato roots (Bleeker *et al.*, 2011). (E)-beta-Ocimene synthase produces a monoterpene released upon herbivory, mechanical wounding, or jasmonic acid treatment (Faldt *et al.*, 2003). Cytokinin induction of these defence genes supports previous studies of cytokinin eliciting a defence response and the defence response being linked to the cytokinin signalling pathway (Choi *et al.*, 2010; Naseem *et al.*, 2012), although the underlying molecular mechanism

Table 1. Hormone-regulated genes commonly induced in all root tissues. Shown are the $\log_2$ fold-change values ($\log_2$FC) for genes commonly induced (>2.0) by cytokinin and auxin among root tip (RT), lateral root (LR), and whole root (WR) with adjusted *P*-values ≤0.05 in one or more tissue.

GeneID	Description	RT		LR		WR	
		$\log_2$FC	P_{adj}	$\log_2$FC	P_{adj}	$\log_2$FC	P_{adj}
Induced by cytokinin							
Solyc01g088160	Sl*CKX2*	2.58	0.046321	3.14	0.00771	3.32	0.003426
Solyc04g080820	Sl*CKX4*	2.90	8.56E-06	3.04	2.12E-06	2.18	0.000725
Solyc12g008900	Sl*CKX6*	5.39	0.027127	7.31	0.00434	7.32	0.002308
Solyc02g071220	Sl*RRA2*	2.31	3.48E-05	3.18	7.98E-09	3.03	2.01E-08
Solyc06g048930	Sl*RRA6*	2.53	0.117332	2.90	0.05133	3.85	0.007115
Solyc06g048600	Sl*RRA7*	2.55	0.000164	2.88	2.00E-05	3.17	2.25E-06
Solyc09g092470	*TPS14*	6.22	2.07E-26	5.21	1.26E-22	3.85	2.75E-14
Solyc01g105960	(E)-beta-ocimene synthase	4.42	9.02E-14	3.61	2.66E-11	3.08	3.86E-09
Solyc02g080790	Sl*DHS*	3.45	0.000293	6.05	4.94E-10	5.69	2.61E-09
Solyc06g076760	Laccase 1a	3.75	8.39E-07	2.27	0.002648	2.63	0.000348
Solyc06g073580	1-aminocyclopropane-1-carboxylate oxidase 1	2.68	0.016622	2.81	0.014623	2.47	0.022694
Solyc12g008740	1-aminocyclopropane-1-carboxylate synthase	3.02	8.96E-12	4.26	1.15E-14	3.21	1.38E-08
Solyc04g010330	Auxin-regulated protein	2.18	0.351047	2.73	0.163185	3.78	0.02978
Solyc02g080120	Gibberellin 2-beta-dioxygenase 7	3.06	6.76E-05	3.16	5.34E-06	2.37	0.000459
Solyc01g059950	GAI-like protein 1	4.87	2.53E-27	3.50	2.87E-14	2.37	1.25E-08
Solyc06g082770	LOB domain family protein	2.60	1.16E-06	3.34	4.00E-18	2.45	7.70E-10
Solyc12g100150	LOB domain protein 4	4.34	1.24E-05	4.84	2.39E-07	5.99	5.71E-09
Induced by auxin							
Solyc01g107400	Indole-3-acetic acid (IAA)-amido synthetase	3.15	1.24E-06	2.20	0.001216	2.11	0.003209
Solyc02g064830	Sl*GH3–3*	7.31	8.65E-107	6.61	4.49E-98	8.22	1.03E-108
Solyc02g092820	Sl*GH3–4*	6.56	1.56E-55	6.76	2.66E-57	7.16	3.70E-59
Solyc07g063850	Sl*GH3–9*	4.95	7.98E-34	4.93	7.20E-33	4.51	2.70E-28
Solyc06g084070	Sl*IAA2*	2.57	3.34E-11	3.64	7.49E-20	3.31	9.31E-16
Solyc12g096980	Sl*IAA5*	3.99	3.01E-45	3.53	1.50E-38	3.45	1.85E-36
Solyc06g008590	Sl*IAA10*	3.78	7.08E-05	3.64	0.000265	2.99	0.004369
Solyc03g120380	Sl*IAA11*	4.91	1.47E-32	5.69	8.86E-37	5.60	3.68E-29
Solyc07g008020	Sl*IAA19*	7.58	1.41E-50	6.92	7.03E-46	7.17	2.95E-41
Solyc08g021820	Sl*IAA21*	5.47	9.70E-09	5.06	2.01E-07	4.26	1.09E-05
Solyc09g083280	Sl*IAA23*	2.22	2.62E-25	2.50	7.22E-31	2.45	6.08E-29
Solyc01g010970	Sl*AGO7*	2.35	1.05E-16	2.24	5.98E-15	2.02	1.23E-12
Solyc12g096110	Protein BREVIS RADIX	4.19	9.56E-33	3.67	1.42E-29	3.52	1.92E-20
Solyc07g048000	Phototropic-responsive NPH3	3.29	4.54E-15	2.97	2.15E-11	2.27	6.34E-07
Solyc03g044090	Phototropic-responsive NPH3 family protein	2.44	1.70E-10	2.74	3.04E-12	2.43	1.67E-09
Solyc04g072300	Ethylene-responsive transcription factor 4	3.49	5.57E-05	4.52	9.39E-07	3.72	0.000107
Solyc05g050830	Ethylene-responsive transcription factor 4	5.99	3.07E-09	6.17	4.36E-09	4.82	3.81E-06
Solyc03g007460	Sl*CRF4*	2.63	5.25E-21	2.70	1.31E-22	2.36	1.04E-15
Solyc01g079260	WRKY transcription factor 4	2.22	3.11E-23	2.07	5.77E-20	2.21	2.02E-21
Solyc11g017100	GRAS family transcription factor	3.27	0.000112	3.54	5.35E-05	2.12	0.026739

behind this connection in tomato roots is obscure and needs further investigation.

Genes associated with abiotic environmental stresses were differentially induced, such as deoxyhypusine synthase Sl*DHS* and six laccase genes: Solyc06g076760 among RT, LR, and WR; Solyc05g052360, Solyc05g052370, and Solyc06g082240 in RT; Solyc05g052400 in LR; and Solyc03g083900 in WR (Table 1 and Supplementary Table S2). DHS is an enzyme that catalyses enzymatic reactions leading to the activation of a eukaryotic translation initiation factor-5A (eIF-5A) (Park *et al.*, 1993, 1997). One study has shown that transcript levels of Sl*DHS* increased due to osmotic stress and chilling injury

Table 2. Experimental validation of a subset of cytokinin/auxin-regulated transcripts. Shown are the $\log_2$ fold-change values from qPCR versus RNA sequencing in root tip (RT), lateral root (LR), and whole root (WR) for genes induced by cytokinin (A) or auxin (B) and repressed by cytokinin and auxin (C).

Gene ID	Description	Tissue	log2 fold-change	
			mRNA sequencing	qPCR
(A) Genes induced by cytokinin				
Solyc04g078900	Cytochrome P450	RT	4.67	4.71
		LR	5.66	3.56
		WR	7.00	2.65
Solyc12g008900	SlCKX6	RT	5.39	4.37
		LR	7.30	3.67
		WR	7.31	3.39
Solyc01g111900	O-Methyltransferase	RT	6.69	5.54
		LR	4.78	4.88
		WR	4.12	3.83
Solyc01g090810	Expansin	RT	3.88	4.22
		LR	3.76	3.12
		WR	5.97	5.51
Solyc12g100150	LOB domain	RT	4.34	4.61
		LR	4.84	4.28
		WR	5.98	3.99
(B) Genes induced by auxin				
Solyc01g090810	Expansin	RT	7.27	6.54
		LR	7.32	6.39
		WR	5.16	4.74
Solyc03g120380	SlIAA11	RT	4.91	4.76
		LR	5.68	5.08
		WR	5.60	4.65
Solyc07g008020	SlIAA19	RT	7.58	7.41
		LR	6.92	7.62
		WR	7.16	6.87
Solyc03g120060	Cytochrome P450	RT	7.85	7.61
		LR	6.80	7.65
		WR	5.70	5.68
Solyc02g064830	SlGH3-3	RT	7.30	8.11
		LR	6.60	8.06
		WR	8.22	6.88

(C) Genes repressed by cytokinin and auxin			Cytokinin		Auxin	
			mRNA sequencing	qPCR	mRNA sequencing	qPCR
Solyc12g009650	Proline-rich protein	RT	−2.98	−3.69	−7.32	−4.98
		LR	−2.46	−2.17	−4.81	−2.60
		WR	−3.26	−2.75	−3.51	−2.47
Solyc01g094910	Ferric reductase oxidase	RT	−4.17	−2.15	−5.72	−3.24
		LR	−5.50	−2.71	−6.30	−2.83
		WR	−6.54	−3.12	−5.05	−3.50

plants (O'Malley *et al.*, 1993). In maize roots, Zm*LAC1*, was shown to be induced by NaCl (Liang *et al.*, 2006). A similar increased transcript level was observed for a tomato laccase gene by salt that were even more abundantly produced when treated with both salt and ABA, likely suggesting an involvement in stress response (Wei *et al.*, 2000). Interestingly, in maize, it has been proposed that apoplastic laccases may have a role in cytokinin breakdown (Galuszka *et al.*, 2005). How this group of genes mediates cytokinin response in responding to stress conditions remains unclear.

Several genes involved in different hormone biosynthesis, degradation, and signalling pathways were also induced. This includes the ethylene biosynthesis enzymes 1-aminocyclopropane-1-carboxylate (ACC) synthase (Solyc12g008740) and ACC oxidase 1 (Solyc06g073580) (Lin *et al.*, 2009), an auxin-regulated protein (Solyc04g010330), and two gibberellic acid (GA)-related genes in involved in GA degradation, gibberellin 2-beta-dioxygenase 7 (Solyc02g080120) (Thomas *et al.*, 1999), and GA signalling, a GAI-like protein 1 (Solyc01g059950) (Table 1; Thomas and Sun, 2004). Induction of these other hormone genes by cytokinin indicates the existence of a complex hormonal crosstalk pathway in tomato roots. Additionally two genes of the *LATERAL ORGAN BOUNDARIES* family (Solyc12g100150 and Solyc06g082770) (Table 1) were found to be induced by cytokinin. This gene family has been linked to a range of developmental processes; including lateral root formation, as well as regulation by auxin and even cytokinin in one case, further supporting hormone connections to root development (Majer and Hochholdinger, 2011).

Cytokinin-repressed genes

More than 50 genes were repressed by cytokinin treatment and can be broadly categorized into processes related to metabolism, development, and transport (Supplementary Table S3). There is a wide range of metabolic processes linked to Cytochrome P450 genes of which four were found to be repressed in the root (Nelson and Werck-Reichhart, 2011).

in tomato leaves (Wang *et al.*, 2001) and the induction of Sl*DHS* by cytokinin as reflected in the present data suggests that the hormone possibly has a role in abiotic stress response mediated by DHS.

Laccases are multi-copper-containing glycoproteins that have been proposed to be involved in lignin synthesis in

There were several different types of transporters or channel proteins that were repressed including, two aquaporins and proteins associated with potassium, manganese, and malate (Supplementary Table S3). While these transporters all facilitate the movement of water or important solutes into the root, it is unclear why they would be repressed in the

presence of additional cytokinin. One additionally interest-ing class of genes is the peroxidases, several of which were found as repressed while others were found to be induced by cytokinin. Peroxidases are known to have a variety of roles in developmental- and defence-related processes and the altered regulation of different peroxidases indicates a complex role for cytokinin in their regulation (Passardi *et al.*, 2005).

Auxin-induced genes

From a total of 17,299 differentially expressed genes, there were 107 genes induced by auxin among all root tissues with >2.0 $\log_2$ fold-change, whereas 175, 20, and 35 genes were specifically regulated in RT, LR, and WR tissues respec-tively (Fig. 1). Also, there were 46 commonly regulated genes in RT and LR tissues, 18 between LR and WR tissues, and 15 between RT and WR tissues (Supplementary Table S4). Overall, the number of auxin-induced genes outnumbered the cytokinin-induced genes in the root tip. Interestingly, sev-eral genes induced by auxin were also induced by cytokinin,

including cytokinin oxidase/dehydrogenase, ACC synthase, gibberellin 2-oxidase (same IDs as mentioned above). This supports a range of findings that indicates there is a complex crosstalk among hormones during root growth and develop-ment (Depuydt and Hardtke, 2011).

Among the differentially expressed auxin-induced genes were four GH3 family genes involved in indole-3-acetic acid (IAA)-amido synthetase (Solyc01g107400, Sl*GH3-3*, Sl*GH3-4*, and Sl*GH3-9*) with $\log_2$ fold-change ranging from 2.10–8.22 (Table 1). Of these, Sl*GH3-3*, Sl*GH3-4*, and Sl*GH3-9* were recently found as being group III members of the GH3 family and were three of the five strongest auxin-induced GH3 fam-ily members in etiolated tomato seedlings treated with hor-mone for 1 or 3 h (Kumar *et al.*, 2012), supporting the present finding of the strong auxin induction of these genes. As these genes are involved in controlling auxin homeostasis levels in the plant, conjugating excess active IAA to an inactive form, their induction from the addition of exogenous auxin is not surprising, although their endogenous role in the in root is not well known (Kumar *et al.*, 2012).
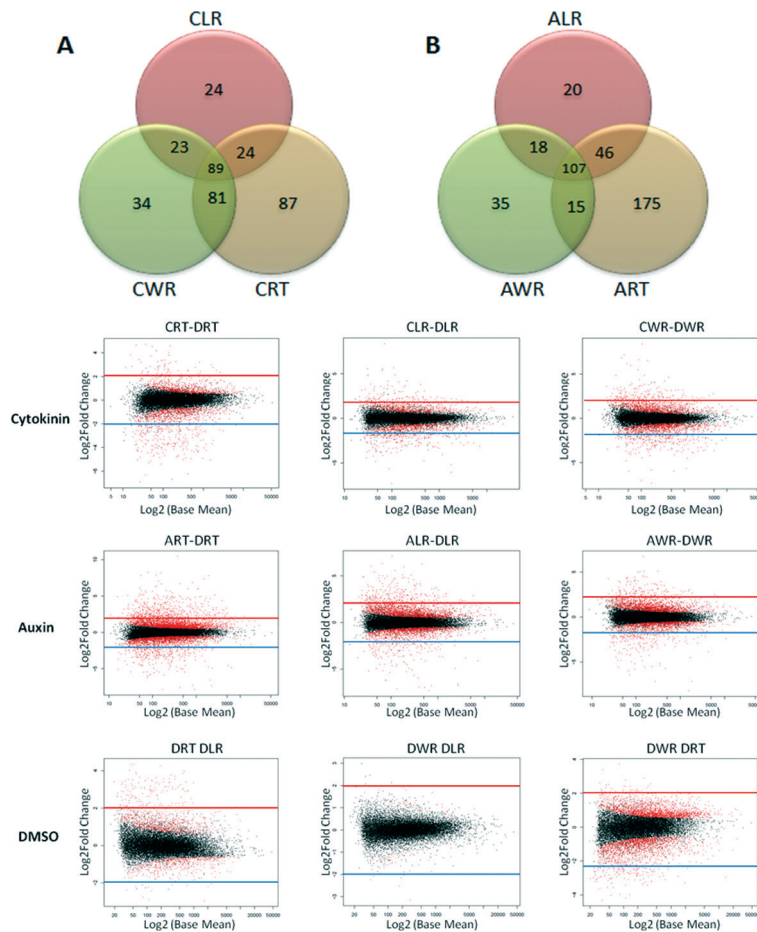

Fig. 1. Differential expression of genes in tomato root tip (RT), lateral root (LR), and whole root (WR). Treatment of root regions are noted as prefixes (C, cytokinin, BA; A, auxin, NAA; D, DMSO). (A, B) Venn diagrams illustrating differentially expressed and significantly induced genes in tomato root either between or unique to specific tissues; gene details are presented in Table 1 and Supplementary Tables 1 and 2. (C) MvA differential expression analysis plots indicating genes' $\log_2$ fold-change versus the $\log_2$ base mean for root tissues treated with cytokinin, auxin, and DMSO control. Genes with >2.0 $\log_2$ fold-change values were identified as differentially regulated as noted in plots by lines (red, induced; blue, repressed). Significantly differentially expressed genes are red dots ($P_{adj} \leq 0.05$)

Auxin induction was also seen in a number of genes belonging to two major auxin-responsive transcriptional regulator families: auxin/indole-3-acetic acid (*Aux/IAA*) and auxin response factor (*ARF*). These gene family members are essential players in auxin signalling, with ARFs acting to activate auxin-regulated targets and Aux/IAA functioning as a transcriptional repressor by binding to ARFs and preventing their transcription factor activity, of which numerous examples have been identified in tomato (Kumar *et al.*, 2011; Wu *et al.*, 2012). In the present study, the Aux/IAA genes Sl*IAA2*, Sl*IAA5*, Sl*IAA10*, Sl*IAA11*, Sl*IAA19*, Sl*IAA21*, and Sl*IAA23* were induced among all root tissues (Table 1), while three ARFs were specifically induced in RT: Sl*ARF2*, Sl*ARF3*, and Sl*ARF9* (Supplementary Table S4). The induction of these Sl*IAA* genes would also be expected in response to the addition of exogenous auxin, in a manner parallel to the induction of Sl*RRAs* by cytokinin, as both are acting to block excess hormone pathway signalling. Interestingly a recent report revealed that Sl*IAA* genes can also be transcriptionally regulated by ethylene in tomato seedlings (Audran-Delalande *et al.*, 2012) suggesting they may function in other signalling pathways and contribute towards complex hormonal crosstalk. Several genetic approaches have been utilized that implicate the importance of *Aux/IAA* and *ARF*s in lateral root formation (De Smet, 2012). While the present study has found that these genes are induced by auxin in the root, they are not found uniquely induced in LR tissues; potentially because LR tissues were not sampled during LR initiation when they are often required.

Auxin also induced Sl*AGO7*, a member of the recently described tomato argonaute (AGO) gene family (Table 1; Bai *et al.*, 2012). Argonaute proteins are components of the RNA-induced silencing complex, which is involved in small RNA-mediated silencing to regulate gene expression. In tomato it was shown that several members of this group were differentially regulated in response to abiotic and viral stresses. Although, Sl*AGO7* in particular was not observed to be differentially regulated by abiotic or viral stress conditions, its induction by auxin provides a new basis for further research. Interestingly miRNAs that are utilized by the RNA-induced silencing complex have been shown to be involved in root cap and LR formation by targeting specific *ARF*s (Wang *et al.*, 2005), and can be regulated by auxin in LR development (Yoon *et al.*, 2010) suggesting a potential role for Sl*AGO7* in this process.

Another gene, *BREVIS RADIX* (*BRX*, Solyc12g096110), which has a role in root development, was induced by auxin (Table 1). *BRX* was first implicated as a regulator of cell proliferation and elongation in the growth zone of root tip (Mouchel *et al.*, 2004), whose protein function is unknown, as far as is known. It has been shown in *Arabidopsis* that *BRX* has a role in cytokinin-mediated inhibition of lateral root initiation which occurred due to loss of auxin response in presumptive founder cells in *brx-2* with indirect involvement of brassinosteroid in the process (Li *et al.*, 2009). How auxin regulation of *BRX* is involved in root development needs to be further explored.

Interestingly, two *NPH3* (non-phototropic hypocotyl 3, Solyc03g044090, and Solyc07g048000) genes were also induced by auxin (Table 1). The function of most *NPH3* family genes is still being investigated; however, recently this group has been linked to the proper localization of PIN protein auxin transporter (Furutani *et al.*, 2011). The addition of exogenous auxin might result in a change in the endogenous auxin transport throughout the root, although this finding does further connect NPHs to auxin-regulated processes.

A range of different transcription factor family genes, which are involved in diverse plant metabolic and developmental roles, were induced by auxin in all root tissues. These include two ERFs (Solyc05g050830 and Solyc04g072300), Sl*CRF4*, a WRKY (Solyc01g079260), and a GRAS family transcription factor (Solyc11g017100) (Table 1). In addition, there was induction by auxin of some transcription factor genes in a root tissue-specific manner. Root tip-specific transcription factors include six MYB family members, an AP2/ERF (Solyc02g092050), heat stress transcription factor A3 (Solyc09g082670), and CYCLOIDEA (Supplementary Table S4). There were two lateral root-specific auxin-induced transcription factors: GATA transcription factor 22 (Solyc01g100220) and Sl*CRF6*. The transcription factor bHLH151 (Solyc06g009510) was induced in WR, but not in the other specific tissues, suggesting that it may be induced in the non-RT or non-LR parts of the primary root (Supplementary Table S4).

Auxin-repressed genes

Auxin was found to repress a larger number of genes, more than 110, compared to the ~50 in cytokinin (Supplementary Table S5). Five different 2-oxoglutarate-dependent dioxygenase (ODDs) class genes with a range of functions from catalysing the production of alkaloids in roots to regulating GA biosynthesis were repressed. A previous report has shown that auxin can inhibit the production of alkaloid production in *Hyoscyamus niger* (Hashimoto *et al.*, 1986) and the present finding would support that link. There are also numerous instances connecting auxin and GA in hormone crosstalk, and since ODDs can function to both activate and inactivate GAs in the biosynthesis pathway, these genes could also support this relationship (Yamaguchi, 2008).

A number of other genes repressed by auxin belong to several of the same gene families or categories that were also repressed by cytokinin, such as CYP p450s, peroxidases, hormone regulation (ACC and GA), transporters (aquaporins and solute/nutrient transporters), genes involved in the biosynthesis of secondary metabolites, and other metabolic and developmental processes (Supplementary Table S5). Other auxin-repressed genes belong to various classes of transcription factors that are involved in protein turnover and stress.

Common genes induced/repressed by both cytokinin and auxin among all root tissues

This study identified several genes that were commonly induced (5) or repressed (28) by cytokinin and auxin in all root three tissues (Supplementary Table S6), which could be broadly categorized into metabolic and developmental

processes. One of the commonly auxin- and cytokinin-induced genes is an expansin (Solyc01g090810), which was further verified as induced by both hormones using qPCR (Table 2). Interestingly this gene is highly related to the beta-expansin *CIM1* from soybean that has previously been shown to be induced by both auxin and cytokinin, indicating that the two hormones can be involved in regulating expansin genes and potentially function to control cell growth in the root (Downes *et al.*, 2001). The present study also confirmed by qPCR the common repression by auxin and cytokinin of ferric reductase oxidase (Solyc01g094910) involved in iron uptake by roots and a proline-rich protein (Solyc12g009650) involved in multiple processes including root nodule formation and response to external stimuli (Sheng *et al.*, 1991; Table 2). The joint regulation of these genes by these two hormones indicates that they likely have vital roles in the root connected to hormone crosstalk.

Transcriptome atlas of tomato root regions: root-tip, lateral root, and whole root

In addition to examining the auxin and cytokinin hormone response in root tissues, this study used differential expression analysis on non-hormone-treated (DMSO) root samples to identify genes expressed in specific regions of the root. Identified were RT, LR, or WR abundantly expressed genes using differential expression comparisons between RT and WR, LR and WR, and RT and LR with the criteria >2.0 $\log_2$ fold-change and $P_{adj} \leq 0.05$ (Supplementary Table S7).

Among the differentially expressed genes in RT, there were several classes of genes involved in defence response, pectin modification, CYP p450, transcription factors (MYB, GATA, and BHLH), root cap proteins, an expansin, nodulin, cell division, and genes involved in other metabolic processes. The differential expression of many of these genes in the RT is not surprising as they are involved in active processes that occur in the RT such as expansion and cell division, as well as the encountering of a variety of biotic/abiotic stresses.

Differentially expressed genes in LR revealed a number of genes that normally play roles in metabolism processes including decarboxylase family protein, UDP-glucosyltransferases, alcohol and aldehyde dehydrogenases, long-chain fatty-acid-CoA ligase, fatty acid elongase 3-ketoacyl-CoA synthase, and ER glycerol-phosphate acyltransferase. In addition to these metabolic genes, a number of auxin-related genes were also differentially expressed in LR, such as Aux/IAAs and SAURs that are known to be linked to LR initiation and development (De Smet, 2012).

Since WR in this study comprised RT and LR, there were common genes expressed between these regions that are involved in metabolism and development. Compared to RT, WR and LR displayed similar profile with more than 120 common genes expressed indicating similarity in functionality of these regions. In addition, there was overrepresentation of genes expressed in WRs related to a range of function, such as ACC oxidases, transport of solute/minerals, CYP p450s, protein turnover, receptor-like kinases, and different transcription factors related to ethylene, auxin, and

cytokinin. Other interesting genes expressed in WRs were three class I heat shock proteins, three water-stress inducible proteins, and two phloem proteins. As these genes cover a wide range of functional classes, it is possible that they may work as individuals or have combinatorial effects for the overall root development in tomato.

In conclusion, in order to identify the genes expressed and/or regulated by the hormones cytokinin and auxin in the tomato root, transcriptome analysis was performed using RNA sequencing. These results present the first genome-wide root tissue analysis of gene response to these hormones. This study reveals numerous significantly novel differentially expressed genes as well as tomato orthologues of auxin- and cytokinin-regulated transcripts. In addition, this RNA sequencing analysis has confirmed hormone regulation of some previously identified tomato auxin- and cytokinin-regulated genes from other studies. Several interactions were identified between different plant hormones and have been discussed in the context of hormonal crosstalk in the root. This study has also generated an atlas of root tissue-specific transcripts and their auxin and cytokinin regulation in whole roots, root tips, and lateral roots. This expression atlas indicates several classes of genes that may have specific roles in different root tissues. Taken together, this study provides a solid foundation of gene expression in tomato roots, both in different tissues and in response to different hormones, which should allow future investigations to more easily study and work to improve tomato roots, plant efficiency, and fruit yield.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Table S1. Primer sequences of genes used for validation of RNA-seq results by quantitative real-time PCR.

Supplementary Table S2. Differentially induced genes >2.0 $\log_2$ fold-change between and among RT, LR, and WR by cytokinin.

Supplementary Table S3. Differentially repressed genes >2.0 $\log_2$ fold-change between and among RT, LR, and WR by cytokinin.

Supplementary Table S4. Differentially induced genes >2.0 $\log_2$ fold-change between and among RT, LR, and WR by auxin.

Supplementary Table S5. Differentially repressed genes >2.0 $\log_2$ fold-change between and among RT, LR, and WR by auxin.

Supplementary Table S6. Differentially induced/repressed genes by both cytokinin and auxin.

Supplementary Table S7. Differentially expressed genes in RT, LR, and WR identified from control (DMSO) samples.

Supplementary Fig. S1. Principal component analysis.

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