Comprehensive Comparison of Auxin-Regulated and Brassinosteroid-Regulated Genes in Arabidopsis[w]

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Although numerous physiological studies have addressed the interactions between brassinosteroids and auxins, little is known about the underlying molecular mechanisms. Using an Affymetrix GeneChip representing approximately 8,300 Arabidopsis genes, we studied comprehensive transcript profiles over 24 h in response to indole-3-acetic acid (IAA) and brassinolide (BL). We identified 409 genes as BL inducible, 276 genes as IAA inducible, and 637 genes in total. These two hormones regulated only 48 genes in common, suggesting that most of the actions of each hormone are mediated by gene expression that is unique to each. IAA-up-regulated genes were enriched in genes regulated in common. They were induced quickly by IAA and more slowly by BL, suggesting divergent physiological roles. Many were early auxin-inducible genes and their homologs, namely *SAUR*, *GH3*, and *IAA*. The comprehensive comparison also identified IAA- and BL-specific genes, which should help to elucidate the specific actions of each hormone. The identified genes were classified using hierarchical clustering based on the similarity of their responses to the two hormones. Gene classification also allowed us to analyze the frequency of cis-elements. The TGTCTC element, a core element of the previously reported auxin response element, was not enriched in genes specifically regulated by IAA but was enriched in the 5′-flanking region of genes up-regulated by both IAA and BL. Such gene classification should be useful for predicting the functions of unknown genes, to understand the roles of these two hormones, and the promoter analysis should provide insight into the interaction of transcriptional regulation by the two hormones.

Auxins play critical roles in the major growth responses during plant development. At the cellular level, auxin acts as a signal for division, expansion, and differentiation throughout the plant life cycle. At the level of the whole plant, auxin plays an important role in root formation, apical dominance, the tropic response, and senescence. By contrast, less attention has been directed to brassinosteroids (BRs) since Grove et al. (1979) isolated the first BR, brassinolide (BL), from oilseed rape (canola [*Brassica napus*]) in 1979. BRs promote stem elongation and inhibit root elongation in various plant species. Nevertheless, only a small number of researchers accepted the hormonal status of BRs before BR mutants were discovered (Clouse, 1996, 1997, 2002) because BRs have activity similar to that of other plant hormones, especially auxins. BRs also interact synergistically with auxin in hypocotyl elongation in several plant species (for review, see Sasse, 1999) and in ethylene production (Arteca et al., 1988). Several authors have proposed that BR-induced effects are mediated via auxin, with BR treatment altering the levels of endogenous auxin or enhancing the sensitivity to auxin (Mandava, 1988; Sasse, 1999). Although numerous physiological studies have addressed the interactions between BRs and auxins, little is known about the underlying molecular mechanisms. Clouse and colleagues made extensive comparisons of the physiological effects of BRs and auxins and conducted molecular analyses of auxin-inducible genes and auxin-insensitive mutants in soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), and Arabidopsis. In soybean and tomato, members of the small auxin up RNAs (*SAUR*) and *GH3* gene families are not induced rapidly during BR-promoted cell expansion but are induced by BR subsequently, even after the beginning of cell elongation, with different kinetics than those induced by auxin treatment (Clouse et al., 1992; Zurek et al., 1994). Mass spectrometry analysis of the free indole-3-acetic acid (IAA) levels in BR-treated tissues showed that the free IAA levels decreased in BR-treated soybean epicotyls (Zurek et al., 1994). It was concluded that BR does not stimulate *SAUR* gene transcription via increased IAA levels. The auxin-insensitive tomato mutant *dgt* (Zurek et al., 1994) and the Arabidopsis mutant *axr1-3* (Clouse et al., 1993) are sensitive to BR. Several studies have concluded that auxin signaling pathways are unlikely to mediate the promotion of cell elongation in soybean and tomato by BR or the inhibition of root elongation in Arabidopsis by BR (Clouse et al., 1992, 1993; Zurek et al., 1994). Conversely, McKay et al. (1994) reported that IAA levels are reduced in the youngest internodes of the pea (*Pisum sativum*) BR-insensitive mutant *lka* and

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the BR-deficient mutant *lkb* (Nomura et al., 1997) as compared with the wild type (WT) using mass spectrometry. This suggested that the endogenous BRs might increase the endogenous IAA content. Therefore, the mechanism of the interaction of these two hormones is still controversial.

The microarray technique is a powerful tool for obtaining an overview of hormone actions using inducible genes as molecular markers. Recently, several microarray studies have examined auxin- and BR-regulated genes. Tian et al. (2002) showed how SHY2/IAA3 affects the expression of auxin-related genes using IAA or mock-treated WT and *shy2* mutants and identified 100 auxin-regulated genes 2 h after IAA treatment. We identified IAA-responsive genes at 15 min (Sawa et al., 2002) and suggested that auxin signals are mediated by a set of diverse transcription factors. BR-responsive genes have also been examined in comprehensive studies of BR-regulated genes in *CPD* antisense, *dwf1-6* (Müssig et al., 2002), *det2*, and *bri1* (Goda et al., 2002) mutants shortly after BR treatment or in comparison with WT plants. These genes have also been studied in the characterization of *bes1-D*, which shows constitutive BR response phenotypes (Yin et al., 2002a). Interestingly, all three reports revealed quick up-regulation of the early auxin-inducible genes in response to BR and indicated overlap of auxin- and BR-regulated genes. By contrast, BRs did not induce all of the early auxin-inducible genes within 3 h (Goda et al., 2002), and it was not clear whether these BR-insensitive auxin-responsive genes (referred to here as auxin-specific genes) are induced subsequently with BR treatment or vice versa (i.e. auxin-insensitive BR-response genes [referred to here as BR-specific genes] are induced subsequently with auxin treatment). To our knowledge, no study has attempted to compare the genes responding to these two hormones comprehensively or the comprehensive time-course response to either of these hormones. To reveal the relationship between auxin and BR actions, we studied the time course of auxin- and BR-regulated genes using an Affymetrix GeneChip representing 8,300 Arabidopsis genes. The results allowed the most comprehensive comparison of auxin- and BR-responsive gene expression to date under the same experimental conditions. This paper presents the kinetics of the auxin and BR responses using responsive genes as molecular markers, revealing the common and distinct actions of these two hormones.

RESULTS AND DISCUSSION

Identification of IAA-Regulated or BR-Regulated Genes

Previously, we showed that BR-regulated genes generally respond to BL in a similar fashion in the WT and a BR-deficient mutant, *det2*, but that the *det2* response to BL is stronger than that of the WT (Goda et al., 2002). Consequently, we used *det2* here and exposed plants to 10 nM BL for up to 24 h to identify BL-regulated genes. Conversely, we exposed WT (Colombia) Arabidopsis to 1 μM IAA for up to 24 h to identify IAA-regulated genes. Transcript abundance was then compared with the mock-treated samples at each time point using the Affymetrix Arabidopsis Genome Array, which represents about 8,300 genes. Hybridization was performed with biotin-labeled cRNA samples prepared from different plant samples in independent hormone-treatment experiments. The signal log ratio (SLR), which is the ratio of the hybridization signals of mock- and hormone-treated plants on a log scale (base 2), was calculated using Affymetrix Microarray Suite (MAS) version 5.0 software. An SLR of 1, for example, indicates a 2-fold increase in the transcript level, and -1 indicates a 2-fold decrease. We extracted genes with expression ratios greater than 2 (i.e. SLR < -1 or SLR > 1) as compared with the mock treatment at each time point. We also used Detection and Change values calculated with MAS to exclude false-positive signals resulting from cross-hybridization or noise. Considering SLR and these two parameters, genes that were reproducibly regulated by BL or IAA in independent experiments were identified as BL- or IAA-regulated genes, respectively. These genes were divided into three groups: genes specifically regulated by IAA (Table I), genes specifically regulated by BL (Table II), and genes regulated by both BL and IAA (Table III). They are listed with the mean and SE of SLR both before and after signal amplification with phycoerythrin-streptavidin (Supplemental Tables I–III; available at www.plantphysiol.org). We classified genes that responded to hormone treatment within 3 h as early inducible genes (including moderate genes) and those responding after 3 h as late inducible genes. The genes were further classified by the direction (up and down) and time (early, late, or both) of their response. Consequently, we extracted 409 BL-inducible genes, 276 IAA-inducible genes, and 637 genes in total.

Overview and Comparison of IAA and BL Induction

The expression profiles of the time-course experiments with the IAA or BL treatments were analyzed by hierarchical clustering (Eisen et al., 1998) using 637 BL- or IAA-regulated genes (listed in Tables I–III). The expression levels are indicated using color (Fig. 1A). The dendrogram represents the relationships between genes based on the similarity of their responses to the two hormones. In the dendrogram, the genes are roughly clustered into four groups (from left to right in Fig. 1A): those up-regulated by IAA (groups A–C), down-regulated by BL (groups D–G), down-regulated by IAA (groups G–I), and up-regulated by BL (groups I–N). The classification in Tables I to III is shown at the bottom of Figure 1A (red, green, and yellow). This classification is not necessarily consistent with the classification using the hierarchical clustering since the genes were classified in the tables using the criteria described above, whereas in the clustergram they were

Table I. *Genes specifically regulated by IAA*

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
Genes Down-Regulated at Early Stage			
19084_AT	At2g07400	Putative retroelement pol polyprotein	A
20675_AT	At2g20750	β-expansin At-EXPB1	H
13463_AT	At4g33790	Male sterility 2-like protein	H
20007_AT	At4g18610	Putative protein	H
20420_AT	At4g19810	Putative chitinase	J
19008_S_AT	At2g28470	Putative β-galactosidase	J
19942_AT	At1g08190	Vacuolar assembly protein vps41, putative	J
20555_S_AT	At4g12280	ACC synthase (AtACS-6)	J
19847_S_AT	At4g19030	Putative water channel	J
14111_S_AT	At4g13900	Putative disease resistance protein	L
14626_AT	At5g35840	Phytochrome C (sp P14714)	L
17331_AT	At4g02420	Ser/Thr kinase-like protein	L
Genes Down-Regulated at Early and Late Stages			
14076_AT	At2g20520	Putative pollen surface protein	G
19672_AT	At1g43160	AP2 domain containing protein, putative	I
Genes Down-Regulated at Late Stage			
16832_AT	At1g05660	Putative polygalacturonase	G
20045_AT	At2g33790	Putative extensin	G
20269_AT	At2g45220	Putative pectinesterase	G
16630_S_AT	At4g25820	XTR9	G
19294_AT	At4g28850	XTR18	G
12365_AT	At4g37160	Pectinesterase-like protein	G
15208_AT	At4g40090	Arabinogalactan-protein (AGP3)	G
16489_AT	At5g46900	Extensin-like protein	G
18533_AT	At5g65730	XTR10	G
15954_AT	At1g66270	β-glucosidase	G
17100_S_AT	At2g32300	Putative uclacyanin I	G
12746_I_AT	At4g11320	Cys proteinase-like protein	G
19348_AT	At4g26220	Caffeoyl CoA *O*-methyltransferase-like protein	G
13686_AT	At2g44110	Similar to Mlo proteins from *Hordeum vulgare*	G
14013_AT	At4g11210	Putative disease resistance protein	G
14550_AT	At4g23690	Putative disease resistance response protein	G
12463_AT	At4g29690	Nucleotide pyrophosphatase-like protein	G
12933_R_AT	At4g33720	Pathogenesis-related protein 1 precursor	G
20442_I_AT	At1g16410	CYP79F1	G
14366_AT	At1g67110	CYP709A2	G
18852_AT	At2g25160	CYP82F1	G
17932_AT	At1g05250	Class III peroxidase PER2	G
17102_S_AT	At1g05260	Class III peroxidase PER3	G
19621_AT	At5g42180	Class III peroxidase PER64	G
19622_G_AT	At5g42180	Class III peroxidase PER64	G
15101_S_AT	At3g14940	Phosphoenolpyruvate carboxylase (PEPC)	G
18743_F_AT	At5g07690	Putative transcription factor	G
17572_S_AT	At1g64780	Ammonium transport protein (AMT1)	G
17571_AT	At3g24300	Ammonium transporter	G
16229_AT	At4g12030	Putative transport protein	G
15666_AT	At5g59520	Putative zinc transporter ZIP2	G
19737_AT	At1g01580	Putative protein	G
14901_AT	At1g62280	Putative protein	G
12758_AT	At2g01530	Putative protein	G
12021_AT	At2g25260	Putative protein	G
15137_S_AT	At2g44790	Putative protein	G
18814_AT	At2g45750	Putative protein	G
19911_AT	At2g48080	Putative protein	G
17748_AT	At4g20460	UDP-glucose 4-epimerase-like protein	H
20524_AT	At1g62560	Similar to flavin-containing monooxygenase (sp P36)	H
13623_R_AT	At4g20820	Reticuline oxidase-like protein	H
15775_AT	At4g29740	Cytokinin oxidase (CKX4)	H
14932_AT	At2g01880	Putative purple acid phosphatase	H
19068_I_AT	At1g14185	Putative protein	H
20446_S_AT	At1g05570	Putative glucan synthase	I
20448_AT	At4g00680	Putative actin-depolymerizing factor	I
15049_AT	At4g02270	Extensin-like protein	I
17485_S_AT	At4g16260	β-1,3-glucanase class I precursor	I
20431_AT	At4g22460	Extensin-like protein	I
20597_AT	At1g53940	Fatty acids and isoprenoids lipase-like protein	I

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
20341_AT	At2g29750	Putative flavonol 3-*O*-glucosyl-transferase	I
13685_AT	At1g61560	Mlo protein, putative	I
16048_AT	At1g73330	Dr4 (protease inhibitor)	I
18983_S_AT	At4g12520	pEARLI 1-like protein	I
16045_AT	At4g15390	HSR201-like protein	I
20367_S_AT	At1g30870	Class III peroxidase PER7	I
18150_AT	At2g39040	Class III peroxidase PER24	I
16971_S_AT	At3g01190	Class III peroxidase PER27	I
15562_AT	At4g26010	Class III peroxidase PER44	I
12400_AT	At5g19890	Class III peroxidase PER59	I
20296_S_AT	At5g67400	Class III peroxidase PER73	I
18459_AT	At4g40010	Putative protein kinase	I
16005_AT	At4g17340	Membrane channel-like protein	I
12004_AT	At4g35060	Farnesylated protein (ATFP6)	I
18888_AT	At1g15380	Putative protein	I
16016_AT	At2g01520	Putative protein	I
20514_I_AT	At2g15370	Putative protein	I
20176_AT	At2g36100	Putative protein	I
20698_AT	At2g40330	Putative protein	I
19195_AT	At2g44380	Putative protein	I
15021_AT	At4g25220	Putative protein	I
16873_I_AT	At2g32530	Putative cellulose synthase	K
19374_AT	At2g28670	Putative disease resistance response protein	K
12139_AT	At4g13580	Putative protein	K
13538_AT	At4g20780	Calcium-binding protein-like	K
16483_AT	At5g65210	Putative transcription factor	K
12341_S_AT	At4g20110	Vacuolar sorting receptor-like protein	K
20180_AT	At4g26320	Putative protein	K
19592_AT	At3g49960	Class III peroxidase PER35	N
20366_AT	At5g22410	Class III peroxidase PER60	N
15851_I_AT	At2g27370	Putative protein	N
Genes Up-Regulated at Early Stage			
20488_AT	At4g34770	SAUR-1	B
14032_AT	At4g37370	CYP81D8	C
12891_AT	At4g11280	ACC synthase (AtACS-6)	C
19409_AT	\NULL	IAA5	C
18216_AT	At1g27730	Putative zinc finger protein	C

Table I. (*Continued*)

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
17303_S_AT	At2g38470	Putative WRKY-type DNA binding protein	C
14711_AT	At2g40140	Putative zinc finger protein	C
16539_S_AT	At4g17490	Ethylene-responsive element binding factor (AtERF6)	C
15288_AT	At2g42430	Putative protein	C
17573_AT	At1g70940	Auxin transport protein REH1	D
16610_S_AT	At1g19050	Putative protein	D
15665_AT	At5g04340	Putative C2H2 zinc finger transcription factor	F
19695_AT	At4g38840	SAUR-14	K
16712_AT	At2g35710	Putative glycogenin	M
20144_AT	At4g25390	Putative protein kinase	M
18258_AT	At2g18210	Putative protein	M
Genes Up-Regulated at Early and Late Stages			
20297_AT	At1g05680	Putative indole-3-acetate β-glucosyltransferase (UGT74E2)	A
16278_AT	At2g37030	SAUR-46	A
17107_AT	At2g22810	1-aminocyclopropane-1-carboxylate synthase (ACS4)	B
13661_AT	At1g52830	IAA6	B
16807_AT	At2g34650	Putative protein kinase	B
14112_AT	At2g41820	Putative protein kinase	B
13439_AT	At4g22780	Translation factor EF-1α-like protein	B
12553_AT	At2g14960	AtGH3-1	C
13293_S_AT	At2g33310	IAA13	C
13297_AT	At3g23030	IAA2	C
13289_S_AT	At4g14560	IAA1	C
16989_AT	At4g27260	AtGH3-5	C
13291_AT	At4g28640	IAA11	C
16878_AT	At1g51170	Putative protein kinase	C
18950_AT	At5g47370	HAT2	C
12090_AT	At2g39370	Putative protein	C
16381_AT	At2g42800	Putative protein	C
Genes Up-Regulated at Late Stage			
20265_AT	At1g22880	Putative endo-1, 4-β-glucanase	A
17268_AT	At2g43860	Putative polygalacturonase	A
18912_AT	At4g13210	Pectate lyase-like protein	A
16974_AT	At4g15160	Cell wall protein-like	A
14346_AT	At4g25240	Pollen-specific protein precursor-like	A
14356_AT	At5g59370	Actin-4 (ACT4)	A
18930_AT	At1g23730	Putative carbonic anhydrase	A
19840_S_AT	At1g30720	Putative reticuline oxidase-like protein	A

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
13406_AT	At2g23540	Putative GDSL-motif lipase/hydrolase	A
19640_AT	At2g29460	Putative glutathione *S*-transferase	A
16843_AT	At2g44460	Putative β-glucosidase	A
17326_AT	At2g44570	Putative glucanase	A
17428_AT	At4g37870	Phosphoenolpyruvate carboxykinase (ATP)-like protein	A
18201_AT	At2g19990	Pathogenesis-related protein (PR-1)	A
18151_AT	At2g35770	Putative Ser carboxypeptidase II	A
19355_S_AT	At2g41280	Late embryogenesis abundant M10 protein	A
14893_AT	At5g12330	Lateral root primordia (LRP1)	A
16993_AT	At5g58860	CYP86A1	A
18960_AT	At1g68850	Class III peroxidase PER11	A
16481_S_AT	At2g18980	Class III peroxidase PER16	A
12355_AT	At2g35380	Class III peroxidase PER20	A
13662_AT	At3g23050	IAA7	A
12444_S_AT	At1g04310	Putative ethylene receptor (ERS2)	A
18908_I_AT	At2g04160	Subtilisin-like Ser protease AIR3	A
20462_AT	At3g13380	Putative protein kinase	A
20343_S_AT	At1g34670	Myb-related protein, putative	A
20424_AT	At2g47260	Putative WRKY-type DNA binding protein	A
20143_AT	At4g30080	Putative transcription factor	A
20720_AT	At1g22990	Putative metal-binding protein	A
17041_S_AT	At3g51895	Sulfate transporter ATST1	A
12083_AT	At1g23060	Putative protein	A
20646_AT	At1g77000	Putative protein	A
19025_AT	At1g77280	Putative protein	A
15347_AT	At2g03830	Putative protein	A
19386_AT	At2g22510	Putative protein	A
18405_S_AT	At2g38480	Putative protein	A
19162_AT	At4g16670	Putative protein	A
19415_AT	At4g20390	Putative protein	A
16514_AT	At4g38080	Putative protein	A
18911_AT	At1g04680	Putative pectate lyase A11	B
16867_AT	At2g32610	Putative cellulose synthase	B
12515_AT	At2g39700	Putative expansin At-EXP4	B
12415_AT	At1g49430	Acyl-CoA synthetase, putative	B
15653_S_AT	At1g78970	Lupeol synthase	B

Table I. (*Continued*)

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
18198_AT	At2g45400	Putative flavonol reductase	B
20238_AT	At3g13790	β-fructofuranosidase	B
20239_G_AT	At3g13790	β-fructofuranosidase	B
13210_AT	At1g11000	AtMlo-h1-like protein	B
15720_AT	At2g03200	Putative chloroplast nucleoid DNA binding protein	B
16440_AT	At2g40000	Putative nematode-resistance protein	B
16963_AT	At2g38390	Class III peroxidase PER23	B
17299_S_AT	At4g25420	Gibberellin 20-oxidase (AtGA20ox1)	B
16099_AT	At4g09460	Putative transcription factor	B
19835_AT	At1g59740	Oligopeptide transporter, putative	B
16816_AT	At1g19230	Putative protein	B
19145_AT	At2g28350	Putative protein	B
15046_S_AT	At2g39710	Putative protein	B
20550_AT	At2g47860	Putative protein	B
19564_AT	At3g46810	Putative protein	B
13016_AT	At4g17350	Putative protein	B
15438_AT	At4g22610	Putative protein	B
14410_AT	At4g24140	Putative protein	B
12821_AT	At4g32460	Putative protein	B
20302_AT	At4g13710	Putative pectate lyase A11	C
14267_AT	At1g30760	Putative reticuline oxidase-like protein	C
13793_AT	At4g26790	Putative APG protein	C
17179_AT	At1g49450	En Spm-like transposon protein, putative	C
17249_AT	At2g19970	Putative pathogenesis-related protein	C
20122_AT	At2g23060	Similar to hookless1 (HLS1)	C
20322_AT	At5g14130	Class III peroxidase PER55	C
16247_AT	At2g45420	Putative protein	C
17697_AT	At2g46740	Putative protein	C
19565_AT	At3g02885	Putative protein	C
14828_AT	At4g30850	Putative protein	C
14606_AT	At2g32990	Putative glucanase	D
12115_AT	At4g22470	Extensin-like protein	D
14733_S_AT	At2g39800	δ-1-pyrroline 5-carboxylase synthetase (P5C1)	D
14025_S_AT	At2g04160	Subtilisin-like Ser protease AIR3	D
19743_AT	At1g65680	Pollen allergen	F
16810_AT	At2g41850	Putative polygalacturonase	F
14446_AT	At2g43670	β-1,3-glucanase-like protein	F
15621_F_AT	At2g22240	Putative myoinositol 1-phosphate synthase	F

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Affymetrix No.	Arabidopsis Genome Initiative Identification (AGI ID)	Gene Name or Comment	Group
18447_AT	At2g40370	Putative laccase	F
17028_S_AT	At1g10460	Germin-like protein (GLP7)	F
13603_F_AT	At4g21650	Subtilisin proteinase-like	F
19045_AT	At2g46950	CYP709B2	F
17514_S_AT	At3g23240	Ethylene response factor 1 (ERF1)	F
16234_AT	At1g49960	Permease homolog (AtPER-X)	F
12506_AT	At2g37360	Putative ABC transporter	F
14790_AT	At1g23560	Putative protein	F
13956_AT	At2g38110	Putative protein	F
18428_AT	At4g35420	Putative protein	F
17885_AT	At4g37900	Putative protein	F
12323_AT	At2g43870	Putative polygalacturonase	J
20328_AT	At2g22420	Class III peroxidase PER17	J
12130_AT	At2g44310	Putative protein	J
19602_AT	At1g49570	Class III peroxidase PER10	K
18596_AT	At1g62570	Flavin-containing monooxygenase, putative	L
13048_S_AT	At2g02850	Putative basic blue protein	L
18786_AT	At4g03140	Putative alcohol dehydrogenase	N

classified using similarity in their gene expression patterns (described in "Materials and Methods"). For example, group H included IAA-down- and BL-up-regulated genes. Most of them are listed as genes specifically regulated by IAA in Table I because their response to BL was less than 2 based on the SLR or was not significant based on the results of the MAS version 5 analysis. The most remarkable finding was that the majority of the genes regulated by both BL and IAA (listed in Table III; shown in yellow in the bottom line of Fig. 1A) were included in the cluster of IAA-up-regulated genes, and they were especially enriched in group B. Interestingly, these genes were up-regulated by BL.

This clustergram represented the general trend of BL- and IAA-regulated genes well, i.e. IAA induction was quicker than BL induction for both up- and down-regulated genes. IAA-regulated genes were detected within 15 min, as we reported previously (Sawa et al., 2002), and the number of IAA-regulated genes peaked at 12 h (data not shown). By contrast, no genes responded in a reproducible manner to BL in 15 min, as we reported previously (Goda et al., 2002), and the number of BL-regulated genes increased continuously over time (Fig. 1A). The difference in induction speed with BL and IAA treatment is also conserved in the genes regulated by both BL and IAA. The lag period for BL-induced gene expression may be due to the time needed to induce auxin biosynthesis or to activate auxin sensitivity. If this is the case, the gene expression pattern in response to BL, especially at early time points, may be similar to the IAA response. To test this hypothesis, the relationship between the gene expression patterns at each time point of the BL and IAA treatments was hierarchically calculated using data on the expression of the 637 genes listed in Tables I to III. The dendrogram indicated that the BL and IAA treatments clustered independently (Fig. 2). In each cluster of the BL and IAA treatments, the continuous experiments were related vicinally. These results suggested that BL and IAA treatments induce gene expression independently. Consistent with this finding, only 48 genes (8%) were regulated by both BL and IAA (Table III); the majority of BL- and IAA-inducible genes are regulated by BL or IAA independently. These results suggest that BL regulates plant growth using a set of genes that is independent from IAA for most of its response.

To overview the functional overlap and divergence of BL- and IAA-inducible genes, the genes were classified into 10 categories based on their established or putative functions. The frequencies of BL- and IAA-inducible genes are shown in Figure 1, B to E (the categories are indicated in Fig. 1, D and E). The largest group of early down-regulated BL genes were P450 genes (Fig. 1C), while relatively few P450 genes were in IAA-regulated genes (Fig. 1B). BL induced more signal transduction-related genes (49 genes, 13.9%), especially at the late stage, than did IAA (17 genes, 4.7%; Fig. 1, C compared with B). IAA induced 17 transcription factor genes (12.7%) at 30 min, consistent with our previous report at 15 min (Sawa et al., 2002), whereas BL induced only one gene (1.9%) at the same time. These results may also reflect the different modes of action in the BL and IAA signal transduction systems. There were fewer down-regulated genes than up-regulated genes in both hormone treatments.

Regulation of the *SAUR*, *GH3*, and *IAA* Gene Families

Genes that are induced by auxins within minutes of treatment are referred to as early auxin-inducible genes, and they form three major gene families, namely *SAUR*, *GH3*, and *IAA* (Hagen and Guilfoyle, 2002; Liscum and Reed, 2002). The *SAUR*, *GH3*, and *IAA* genes predominated in IAA-up-regulated genes, both early and late (Fig. 1B). This is consistent with previous DNA-microarray studies of IAA-inducible genes studied at early times (Sawa et al., 2002; Tian et al., 2002). These genes also predominated in BL-up-regulated genes (Fig. 1C). Interestingly, these families are relatively more enriched in genes regulated by both BL and IAA (Fig. 1, E compared with D). Previously, we showed that BL treatment induced

Table II. *Genes specifically regulated by BL*

Affymetrix No.	AGI ID	Gene Name or Comment	Group
Genes Down-Regulated at Early Stage			
20271_AT	At4g37310	CYP81H1	A
14448_AT	At2g45210	SAUR-36	A
17039_AT	At3g26220	CYP71B3	D
18190_AT	At2g46660	CYP78A6	E
16603_S_AT	At4g15550	Indole-3-acetate glucosyltransferase-like protein (UGT75D1)	E
20174_AT	At2g43060	Transcription factor-like protein	E
18780_AT	At2g43440	Transcription factor-like protein	E
17576_AT	At1g23080	PIN7	E
12372_AT	At1g77380	Amino acid carrier	E
16163_S_AT	At4g22200	AKT3	E
18290_AT	At1g49500	Putative protein	E
19977_AT	At3g48360	Putative protein	E
13656_AT	At4g01870	Putative protein	E
18295_S_AT	At1g03880	Putative cruciferin 12S seed storage protein	F
13198_I_AT	At4g28520	12S cruciferin seed storage protein	F
20362_AT	At1g71030	Putative transcription factor	J
Genes Down-Regulated at Early and Late Stages			
16119_S_AT	At2g30070	AtKUP1	D
14240_S_AT	At1g77760	Nitrate reductase 1 (NR1)	E
12998_AT	At3g47800	Aldose 1-epimerase-like protein	E
14856_AT	At2g34490	CYP710A	E
13870_AT	At3g50660	DWF4	E
16535_S_AT	At4g36380	ROT3	E
16042_S_AT	At5g05690	CPD	E
14630_S_AT	At1g09530	PIF3	E
19221_AT	At4g36780	Putative protein	E
19398_AT	At4g37540	Putative protein	E
Genes Down-Regulated at Late Stage			
19684_AT	At4g34970	Actin depolymerizing factor-like protein	A
17795_AT	At2g14050	Putative DNA replication licensing factor	A
15669_S_AT	At1g06570	4-hydroxyphenyl-pyruvate dioxygenase (HPD)	D
15142_AT	At1g22360	UDP-glucose glucosyltransferase	D
19759_AT	At1g23020	Putative superoxide-generating NADPH oxidase flavo	D
15190_S_AT	At2g26740	Epoxide hydrolase (ATsEH)	D
12798_AT	At2g38230	Similar to SOR1 from the fungus *Cercospora nicotia*	D
17002_AT	At3g51600	Nonspecific lipid transfer protein	D
14663_S_AT	At4g24040	Trehalase-like protein	D
12815_AT	At4g27450	Amino acid biosynthesis Gln-dependent Asp synthetase	D
13242_AT	At4g37980	Cinnamyl-alcohol dehydrogenase ELI3-1	D
15144_S_AT	At5g14740	CARBONIC ANHYDRASE 2	D
13824_AT	At5g23310	Iron superoxide dismutase 3	D
19815_AT	At1g14210	Ribonuclease	D
13286_S_AT	At2g04030	Putative heat shock protein	D
20256_S_AT	At2g22990	Proteolysis Ser-type carboxypeptidase-like protein	D
18005_AT	At3g61620	Exonuclease RRP41	D
18699_I_AT	At5g15970	Cold-regulated protein COR6.6 (KIN2)	D
17566_AT	At5g40160	Ankyrin repeat protein EMB506	D
19730_AT	At4g39480	CYP96A9	D
13385_AT	At1g14030	Putative Rubisco oxy	D
15153_AT	At3g27690	Lhcb2 protein (Lhcb2:4)	D
15793_AT	At4g23940	FtsH protease, putative	D
14039_AT	At2g19590	1-aminocyclopropane-1-carboxylate oxidase	D
14557_AT	At1g02280	Putative GTP-binding protein	D
19749_AT	At1g31230	Putative protein kinase	D
16258_AT	At2g39510	Nodulin-like protein	D
16124_S_AT	At2g47590	Photolyase/blue light photoreceptor PHR2 (PHR2)	D
20120_AT	At1g03970	G-box binding factor, GBF4	D
13168_I_AT	At2g45050	Putative GATA-type zinc finger transcription factor	D
19059_AT	At2g47520	Putative AP2 domain transcription factor	D
13642_AT	At1g23180	Nuclear transport AtKAP α	D
18800_AT	At1g60160	Potassium transporter AtKT5p	D
16613_S_AT	At2g40540	Putative potassium transporter	D
17042_S_AT	At4g02700	Sulfate transporter protein	D
12772_AT	At1g03220	Putative protein	D
13868_AT	At1g15440	Putative protein	D
17672_AT	At1g24340	Putative protein	D
19266_AT	At1g47580	Putative protein	D
13680_AT	At1g55020	Putative protein	D
18716_AT	At1g75830	Putative protein	D
13085_I_AT	At1g78820	Putative protein	D
13181_AT	At2g02160	Putative protein	D
12768_AT	At2g15890	Putative protein	D
15702_S_AT	At2g17250	Putative protein	D

(*Table continues on following page.*)

Table II. (*Continued from previous page.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
18396_AT	At2g34640	Putative protein	D
13382_AT	At2g42750	Putative protein	D
13700_AT	At3g04550	Putative protein	D
16637_S_AT	At4g14690	Putative protein	D
20117_AT	At4g16370	Putative protein	D
14476_AT	At4g17940	Putative protein	D
15357_AT	At4g33560	Putative protein	D
13654_AT	At4g39040	Putative protein	D
20615_AT	At2g29390	Putative C-4 sterol methyl oxidase	E
19215_AT	At2g43910	Putative methyl chloride transferase	E
13573_AT	At4g37550	Formamidase-like protein	E
12526_AT	At4g27710	CYP709B3	E
20389_AT	At5g65310	Homeobox-Leu zipper protein ATHB-5 (HD-zip pro)	E
14068_S_AT	At2g36950	Putative farnesylated protein	E
17832_AT	At2g16060	Class I nonsymbiotic hemoglobin (AHB1)	F
16253_AT	At2g17845	Putative protein	F
19826_AT	At1g12040	Putative extensin	G
13449_AT	At4g36700	Globulin-like protein	G
13197_R_AT	At4g27170	Putative protein	G
13278_F_AT	At5g12030	Heat shock protein 17.6A	H
19060_AT	At1g70300	Potassium transporter	H
12340_AT	At1g10450	Putative protein	L
Genes Up-Regulated at Early Stage			
19905_AT	At4g19420	Putative pectinacetylesterase	K
12335_AT	At2g47060	Putative protein kinase	K
19992_AT	At4g01950	Putative protein	K
19142_AT	At1g23030	Putative protein	L
19211_AT	At4g27720	12S cruciferin seed storage protein	N
13812_AT	At4g03400	AtGH3-10	N
15271_AT	At2g34510	Putative protein	N
Genes Up-Regulated at Early and Late Stages			
20689_AT	At2g43290	Putative calcium-binding protein	B
18300_AT	At5g37770	TCH2	B
17961_AT	At1g01120	Fatty acid elongase 3-ketoacyl-CoA synthase 1 (KCS1)	K
17960_AT	At1g65310	XTR1	K
19660_AT	At2g40610	AtExp8	K
14612_AT	At4g02330	Putative pectin methylesterase	K
20537_AT	At4g13340	Extensin-like protein	K
15892_AT	At2g19620	Putative SF21 protein (*Helianthus annuus*)	K
12251_AT	At2g34930	Putative disease resistance protein	K
17966_AT	At4g00360	CYP86A2	K
12356_AT	At5g06720	Class III peroxidase PER53	K
18253_S_AT	At1g76680	12-oxophytodienoate reductase (OPR1)	K
13322_AT	At4g38860	SAUR-16	K

Table II. (*Continued.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
17440_I_AT	At1g78860	Protein kinase	K
12584_AT	At2g44500	Similar to axi 1 protein from tobacco (*Nicotiana tabacum*)	K
19857_AT	At4g31000	Putative calmodulin-binding protein	K
13806_AT	At2g17040	NAM (no apical meristem)-like protein	K
16438_AT	At1g03870	Putative protein	K
12046_AT	At1g30690	Putative protein	K
13916_AT	At2g19800	Putative protein	K
15403_S_AT	At2g31730	Putative protein	K
19880_AT	At2g47440	Putative protein	K
12027_AT	At4g20170	Putative protein	K
14947_AT	At4g37450	Putative protein	K
19976_AT	At4g38400	Putative pollen allergen	N
15107_S_AT	At5g10430	AtAGP4	N
14077_AT	At4g08950	Putative phi-1-like phosphate-induced protein	N
17196_AT	At4g28780	Pro-rich APG-like protein	N
19288_AT	At2g27690	CYP94C1	N
14779_AT	At2g30010	Putative protein	N
Genes Up-Regulated at Late Stage			
18180_AT	At2g15310	Putative ADP-ribosylation factor	A
17338_AT	At2g47550	Putative pectinesterase	B
13706_AT	At2g18700	Putative trehalose-6-phosphate synthase	B
18567_AT	At2g47130	Putative alcohol dehydrogenase	B
18818_AT	At2g12210	Putative TNP2-like transposon protein	B
17403_AT	At2g45550	CYP76C4	B
13209_S_AT	At1g04250	IAA17	B
13022_AT	At1g34750	Protein phosphatase type 2C, putative	B
15085_AT	At4g23010	GOG5-GDP-mannose transporter	B
15556_AT	At1g21820	Putative protein	B
20194_AT	At2g17500	Putative protein	B
20616_AT	At2g32560	Putative protein	B
17262_AT	At2g15510	Putative non-LTR retroelement reverse transcriptase	F
18670_G_AT	At4g17090	Putative β-amylase	H
20277_I_AT	At4g13310	CYP71A20	I
18922_AT	At3g07850	Exopolygalacturonase	J
12514_AT	At4g19750	Chitinase-like protein	J
12508_I_AT	At4g19760	Chitinase-like protein	J
13926_AT	At2g27920	Putative carboxypeptidase	J
16666_AT	At4g32540	Dimethylaniline monooxygenase-like protein	J
18422_AT	At2g01790	Similarity to human ubiquitin-specific protease	J

(*Table continues on following page.*)

Table II. (*Continued from previous page.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
16701_AT	At2g02310	Putative phloem-specific lectin	J
19753_AT	At2g14300	Putative helicase	J
18997_S_AT	At2g23500	Mutator-like transposase	J
12564_AT	At2g30810	Gibberellin-regulated protein homolog	J
20630_I_AT	At2g40290	Putative eukaryotic translation initiation factor	J
12264_I_AT	At4g13610	DNA (cytosine-5-)-methyltransferase-like protein	J
16781_AT	At2g19130	Putative protein kinase	J
16360_AT	At4g21380	Putative protein kinase	J
20262_AT	At1g61140	Potassium transporter AtKT5p	J
19270_AT	At5g23270	Monosaccharide transporter	J
13374_AT	At1g23570	Putative protein	J
19676_AT	At2g22620	Putative protein	J
15859_AT	At2g28570	Putative protein	J
20124_AT	At2g29860	Putative protein	J
15287_S_AT	At3g47280	Putative protein	J
12502_AT	At4g19720	Putative protein	J
17305_AT	At1g53830	Putative pectin methylesterase	K
17386_AT	At2g21140	Extensin-like protein	K
12577_AT	At2g28630	Putative fatty acid elongase	K
12364_AT	At3g57240	β-1,3-glucanase (BG3)	K
18265_AT	At4g12730	Putative pollen surface protein	K
17899_AT	At4g15610	Cell wall protein-like	K
12239_AT	At4g29020	Biogenesis of cell wall (cell envelope) Gly-rich protein	K
16052_AT	At5g23860	β-8 tubulin (TUB8)	K
18968_AT	At5g57550	XTR3 (EXGT-A5)	K
19199_AT	At1g24170	Putative glycosyl transferase	K
16981_S_AT	At1g45145	Thioredoxin, putative	K
12277_AT	At1g47600	Thioglucosidase, putative	K
20391_AT	At2g23560	Putative acetone-cyanohydrin lyase	K
17008_AT	At2g24850	Putative Tyr aminotransferase	K
19129_AT	At2g30670	Putative tropinone reductase	K
16017_AT	At3g16370	Putative APG protein	K
12574_AT	At3g60140	β-glucosidase-like protein	K
20305_AT	At4g01070	Putative flavonol glucosyltransferase	K
16444_AT	At4g13890	Gly hydroxymethyl-transferase-like protein	K
17449_AT	At4g14440	Carnitine racemase-like protein	K

Table II. (*Continued.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
13908_S_AT	At4g20860	Berberine bridge enzyme-like protein	K
12539_S_AT	At4g39640	Putative γ-glutamyl-transferase	K
18250_AT	At5g16990	Quinone oxidoreductase-like protein	K
19178_AT	At5g20230	Blue copper binding protein	K
19339_I_AT	At2g10140	Putative TNP2-like transposon protein	K
14635_S_AT	At2g14610	Pathogenesis-related PR-1-like protein	K
19863_AT	At2g14900	Gibberellin-regulated protein homolog	K
16730_AT	At2g16040	Ac-like transposase	K
13004_AT	At2g17840	Putative senescence-associated protein 12	K
13498_S_AT	At2g32450	Putative *O*-GlcNAc transferase	K
20268_S_AT	At3g46840	Subtilisin-like proteinase	K
16021_AT	At4g20260	Endomembrane-associated protein	K
16482_S_AT	At4g32940	γ-VPE (vacuolar processing enzyme)	K
16465_AT	At5g02490	dnaK-type molecular chaperone hsc70.1-like	K
20278_S_AT	At4g13290	CYP71A19	K
12342_AT	At1g24650	Putative protein kinase	K
20227_S_AT	At1g52030	Myrosinase-binding protein, putative	K
16790_AT	At1g53700	Putative protein kinase	K
19434_AT	At2g04300	Putative protein kinase	K
16393_S_AT	At2g13790	Putative protein kinase	K
12497_AT	At2g31880	Putative protein kinase	K
17752_AT	At2g32800	Putative protein kinase	K
12958_AT	At2g33580	Putative protein kinase	K
12353_AT	At2g37710	Putative protein kinase	K
15475_S_AT	At2g40270	Putative protein kinase	K
17917_S_AT	At2g41090	Calcium-binding protein (CaBP-22)	K
13217_S_AT	At3g50770	Calmodulin-like protein	K
17291_AT	At4g13000	Putative protein kinase	K
17989_S_AT	At4g14640	Calmodulin	K
20232_S_AT	At4g23130	Putative protein kinase	K
20246_S_AT	At4g23250	Putative protein kinase	K
20373_AT	At4g39890	GTP-binding protein GB2	K
17113_S_AT	At5g58670	Phosphoinositide-specific phospholipase C	K
19936_AT	At1g70000	DNA binding protein MybSt1	K
13432_AT	At2g25000	Putative WRKY-type DNA binding protein	K

(*Table continues on following page.*)

Table II. (*Continued from previous page.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
20382_S_AT	At2g30250	Putative WRKY-type DNA binding protein	K
20619_AT	At2g37430	Putative transcription factor	K
14507_AT	At2g38610	Putative RNA-binding protein	K
12471_S_AT	At4g03110	Putative ribonucleoprotein	K
16298_AT	At4g21850	Putative transcription factor	K
13672_AT	At5g11060	HOMEOBOX PROTEIN KNOTTED-1 LIKE 4 (KNAT4)	K
16488_AT	At1g11260	Glucose transporter	K
17278_AT	At1g30900	Vacuolar sorting receptor-like protein	K
19450_AT	At1g71880	Sucrose transport protein SUC1	K
19122_AT	At2g29330	Putative tropinone reductase	K
15987_AT	At2g39010	Putative aquaporin	K
15934_I_AT	At3g01930	HXT6 high-affinity hexose transporter	K
18328_AT	At3g19930	HXT7 high-affinity hexose transporter	K
20369_S_AT	At4g13510	Ammonium transport protein (AMT1)	K
20521_AT	At4g18910	Major intrinsic protein (MIP)-like	K
17451_AT	At4g24120	Putative oligopeptide transporter	K
12943_AT	At1g03370	Putative protein	K
18881_AT	At1g12080	Putative protein	K
12105_AT	At1g22890	Putative protein	K
15338_AT	At1g23840	Putative protein	K
16202_AT	At1g47730	Putative protein	K
20469_AT	At1g60030	Putative protein	K
14964_AT	At1g65500	Putative protein	K
20594_AT	At1g70230	Putative protein	K
15846_AT	At2g14560	Putative protein	K
12642_AT	At2g15390	Putative protein	K
14916_AT	At2g16630	Putative protein	K
19369_AT	At2g17120	Putative protein	K
12392_AT	At2g23290	Putative protein	K
15540_AT	At2g24860	Putative protein	K
19856_AT	At2g25300	Putative protein	K
14924_AT	At2g28400	Putative protein	K
13428_AT	At2g31120	Putative protein	K
16422_AT	At2g33830	Putative protein	K
18287_AT	At2g37940	Putative protein	K
12990_AT	At2g41170	Putative protein	K
19363_AT	At2g42610	Putative protein	K
12084_AT	At2g43340	Putative protein	K
18635_AT	At2g43920	Putative protein	K
12037_AT	At2g44130	Putative protein	K
20017_AT	At2g44290	Putative protein	K
13539_I_AT	At3g47380	Putative protein	K
12171_AT	At3g52500	Putative protein	K
20429_AT	At4g14400	Putative protein	K
14401_AT	At4g15630	Putative protein	K

Table II. (*Continued.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
15815_AT	At4g17070	Putative protein	K
12561_AT	At4g19120	Putative protein	K
14946_AT	At4g21620	Putative protein	K
14431_AT	At4g23810	Putative protein	K
14400_AT	At4g25260	Putative protein	K
12696_AT	At4g26250	Putative protein	K
12209_AT	At4g26950	Putative protein	K
19182_AT	At4g33050	Putative protein	K
12443_AT	At4g34480	Putative protein	K
15084_AT	At4g35320	Putative protein	K
15817_AT	At4g37240	Putative protein	K
13055_AT	At4g38030	Putative protein	K
14882_AT	At4g39670	Putative protein	K
12118_AT	At4g39840	Putative protein	K
16897_I_AT	At5g15350	Putative protein	K
16053_I_AT	At1g02920	Glutathione *S*-transferase, putative	L
17372_AT	At1g62040	Symbiosis-related protein, putative	L
14362_AT	At2g30310	Putative GDSL-motif lipase/hydrolase	L
13014_AT	At2g30550	Putative lipase	L
13977_AT	At2g41540	Glycerol-3-phosphate dehydrogenase	L
19636_AT	At3g25110	Acyl-(acyl carrier protein) thioesterase	L
13942_AT	At3g50760	Glycosyltransferase-like protein	L
19171_AT	At2g43510	Putative trypsin inhibitor	L
19993_AT	At1g78490	CYP708A3	L
15982_S_AT	At2g37130	Class III peroxidase PER21	L
12333_AT	At4g36430	Class III peroxidase PER49	L
16350_AT	At1g61390	Putative protein kinase	L
12276_AT	At2g28960	Putative protein kinase	L
16990_AT	At2g37640	Nodulin-like protein	L
15972_AT	At4g16190	Cys proteinase	L
20346_AT	At4g35600	Putative protein kinase	L
20027_AT	At1g50420	Scarecrow-like 3	L
18933_AT	At2g40300	Putative ferritin	L
16296_AT	At4g04770	Putative ABC transporter	L
16031_AT	At5g01600	Ferritin 1 precursor	L
15032_AT	At1g61250	Putative protein	L
19709_I_AT	At1g62430	Putative protein	L
14959_AT	At1g79450	Putative protein	L
12062_AT	At2g01650	Putative protein	L
19207_AT	At2g01670	Putative protein	L
14381_AT	At2g02810	Putative protein	L
17448_AT	At2g16530	Putative protein	L
14423_AT	At2g25190	Putative protein	L
18267_AT	At2g32210	Putative protein	L
15854_AT	At2g36820	Putative protein	L
13475_AT	At2g38200	Putative protein	L
14972_AT	At2g38740	Putative protein	L
13941_AT	At4g12850	Putative protein	L
12540_AT	At4g14390	Putative protein	L
14825_AT	At4g21240	Putative protein	L

(*Table continues on following page.*)

Table II. (*Continued from previous page.*)

Affymetrix No.	AGI ID	Gene Name or Comment	Group
12995_AT	At4g24970	Putative protein	L
12968_AT	At4g28270	Putative protein	L
15938_AT	At4g33100	Putative protein	L
14884_AT	At4g33910	Putative protein	L
16968_AT	At4g34131	Putative protein	L
15877_AT	At4g35330	Putative protein	L
13059_AT	At4g36550	Putative protein	L
13955_AT	At5g44810	Putative protein	L
16700_AT	At2g02250	Lectin-like protein	M
14355_AT	At1g80390	IAA15	M
18758_AT	At4g17660	Putative protein kinase	M
13659_AT	At4g23150	Putative protein kinase	M
20489_AT	At2g44840	Putative ethylene-response element binding protein (EREBP)	M
19340_S_AT	At4g03900	Putative transposon protein	M
16439_AT	At1g31580	Putative protein	M
18828_AT	At1g55660	Putative protein	M
16199_AT	At1g65160	Putative protein	M
14348_AT	At4g13700	Putative protein	M
15111_S_AT	At2g06850	EXGT-A1(EXT)	N
19017_AT	At4g37800	XTR15	N
16279_AT	At2g04570	Putative GDSL-motif lipase hydrolase	N
14089_AT	At2g32150	Putative hydrolase	N
15601_S_AT	At2g34770	Fatty acid hydroxylase (FAH1)	N
13577_S_AT	At4g24510	CER2	N
16791_AT	At4g39830	Putative L-ascorbate oxidase	N
16365_AT	At2g32680	Putative disease resistance protein	N
13625_S_AT	At3g50950	Putative disease resistance protein	N
13177_AT	At4g12720	Growth factor-like protein	N
19502_AT	At4g39510	CYP96A12	N
13857_AT	At2g21220	SAUR-12	N
16348_AT	At1g65790	Putative protein kinase	N
19290_AT	At2g21540	Putative phosphatidyl-inositol/ phophatidylcholine transfer protein	N
17990_AT	At3g51920	Putative calmodulin	N
12360_AT	At4g23210	Putative protein kinase	N
15779_G_AT	At3g46090	Zinc finger protein ZAT7	N
19750_AT	At2g16960	Putative importin	N
18844_AT	At2g29120	Putative ligand-gated ion channel protein	N
20128_AT	At1g16420	Putative protein	N
19825_AT	At1g65550	Putative protein	N
19065_AT	At2g37440	Putative protein	N
19985_I_AT	At2g47080	Putative protein	N
14249_I_AT	At3g52430	Putative protein	N
20199_AT	At3g52480	Putative protein	N
17653_AT	At4g39030	Putative protein	N

a member from each gene family (*SAUR-AC1*, *GH3-homolog BRU6*, and *IAA3/SHY2*) after a lag period of 30 to 60 min (Goda et al., 2002). This comprehensive study also demonstrated that most genes in this category regulated by both IAA and BL are regulated quickly by IAA but more slowly by BL (Fig. 3A). A possible mechanism for the difference in induction speed is discussed below. The difference in induction speed between the two hormones suggests that auxin regulates rapid physiological responses, such as tropic responses, whereas BR regulates slower physiological responses, such as developmental regulation and more gradual responses to the environment.

Of the three gene families, the *SAUR* genes had the strongest BL responses (SLR > 3) in the BL treatment (Fig. 3B, red lines). By contrast, the *GH3* and *IAA* genes had the strongest IAA responses (Fig. 3B, green and blue lines, respectively). This complementary inducibility may be related to the synergism between BR and auxin. The expression of *SAUR* genes correlates well with auxin-induced elongation (McClure and Guilfoyle, 1987, 1989; Gee et al., 1991), although their functions are still unclear. Yang and Poovaiah (2000) demonstrated that the amino-terminal domain of SAUR proteins binds to calmodulin in maize (*Zea mays*), soybean, and Arabidopsis. Very recently, we demonstrated that the expression of *SAUR-AC1* correlates well with BR-mediated elongation and that it is regulated by BRs independently of the endogenous auxin levels (Nakamura et al., 2003b). These findings, together with our finding that a number of genes encoding calcium-binding protein are regulated by BL or IAA (Tables I–III), suggest that the calcium and calmodulin system is an important target for studying BR and auxin signal interaction.

In this study, we also identified *GH3* and *IAA* genes: IAA specifically regulated eight genes (*AtGH3-1* and *5*, and *IAA1*, *2*, *6*, *7*, *11*, and *13*); BL specifically regulated three genes (*AtGH3-10*, *IAA15*, and *IAA17/AXR3*); and both BL and IAA regulated six genes (*AtGH3-2* and *3*, and *IAA3*, *5*, *19*, and *26*). The *IAA17/AXR3* gene, an auxin-inducible gene (Ouellet et al., 2001), was not identified as an IAA-responsive gene since its response to IAA was below the threshold (SLR = 0.7). Mutants in members of these gene families exhibit phenotypes with insensitivity to auxin and other hormones, as well as defects in light signaling and photomorphogenesis (Hagen and Guilfoyle, 2002; Liscum and Reed, 2002; Swarup et al., 2002). By contrast, we found that BL-induced *IAA* genes in a manner independent of the endogenous IAA level (Nakamura et al., 2003a). These findings suggest that *IAA* and *GH3* genes are important cross talk points in BR, auxin, light, and other signaling pathways.

Regulation of Genes Involved in Cell Expansion or Cell Wall Organization

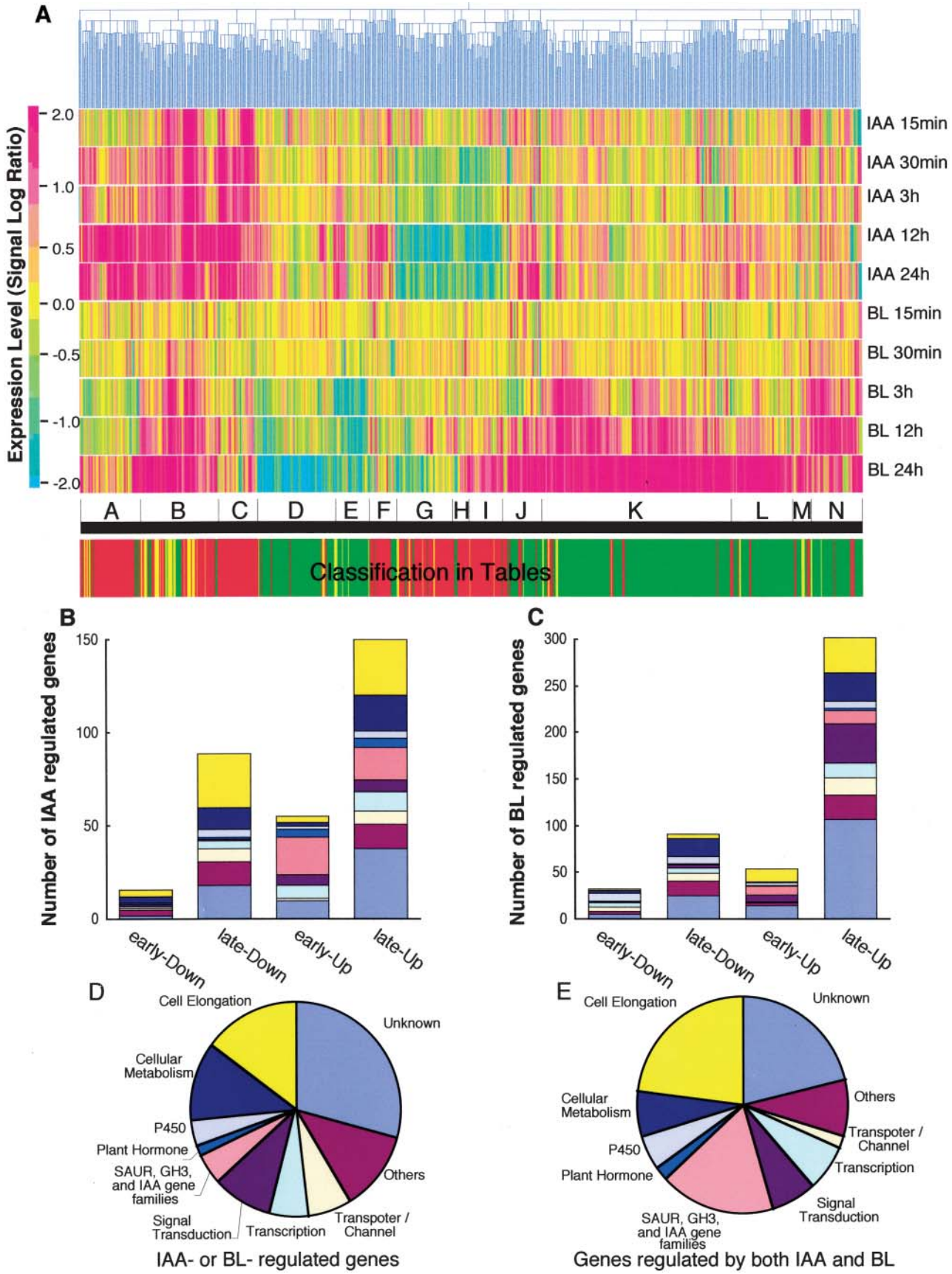
The regulation of tissue elongation is an important function of both BR and auxin. Synergistic interactions

Table III. *Genes commonly regulated by IAA and BL*

Affymetrix No.	AGI ID	Gene Name or Comment	Group
Genes Down-Regulated by IAA and BL			
20547_AT	At5g04950	Nicotianamine synthase	E
17045_AT	At1g78090	Trehalose-6-phosphate phosphatase (AtTPPB)	G
16070_S_AT	At3g60280	Uclacyanin 3 (UCC3)	G
17849_S_AT	At1g09090	Putative respiratory burst oxidase protein B	G
17255_AT	At2g25980	Similar to jasmonate-inducible proteins from Brassica	G
12748_F_AT	At4g11320	Cys proteinase-like protein	G
14117_AT	At4g37410	CYP81F4	G
Genes Up-Regulated by IAA and BL			
18955_AT	At1g04220	Putative β-ketoacyl-CoA synthase	B
13301_AT	At1g04240	IAA3	B
13660_I_AT	At1g15580	IAA5	B
13781_AT	At2g18010	SAUR-10	B
17894_AT	At2g18690	Putative protein	B
16995_AT	At2g23170	AtGH3-3	B
12543_AT	At2g26710	BAS1	B
15005_S_AT	At2g30040	Putative protein kinase	B
12330_AT	At2g34080	Cys proteinase	B
18885_AT	At2g36220	Putative protein	B
13296_AT	At3g15540	IAA19	B
13999_AT	At4g03420	Putative protein	B
14951_AT	At4g09890	Putative protein	B
13395_AT	At4g13790	SAUR-25	B
12501_AT	At4g21200	Gibberellin 2-oxidase (AtGA2ox8)	B
15431_AT	At4g27280	Stress response calcineurin B-like protein	B
12947_AT	At4g36110	SAUR-9	B
13565_AT	At4g37390	AtGH3-2	B
12608_I_AT	At4g38850	SAUR-AC1	B
18946_AT	At5g39580	Class III peroxidase PER62	B
20035_AT	At5g44440	Berberine bridge enzyme-like protein	B
17292_AT	At5g49630	Amino acid permease 6	B
15985_AT	At5g64100	Class III peroxidase PER69	B
20334_S_AT	At1g74650	Putative transcription factor	J
19490_AT	At1g10550	Putative endoxyloglucan transferase	K
16434_AT	At4g18970	Putative protein	K
13495_S_AT	At2g02850	Basic blue protein	L
15933_AT	At1g21830	Putative protein	M
20502_AT	At2g21200	SAUR-7	M
18284_AT	At4g34150	Putative protein	M
17533_S_AT	At4g25810	XTR6	N
16620_S_AT	At5g57560	TCH4	N
Genes Down-Regulated by IAA and Up-Regulated by BL			
16028_AT	At4g30170	Class III peroxidase PER45	I
20608_S_AT	At2g44390	Putative protein	I
12438_AT	At4g18430	Membrane-bound small GTP-binding-like protein	K
18224_S_AT	At4g21830	Putative transcription factor	K
12953_AT	At4g01080	Putative protein	K
Genes Up-Regulated by IAA and Down-Regulated by BL			
19177_AT	At5g22500	Male sterility 2-like protein	A
19281_I_AT	At2g23180	CYP96A1	A
15434_AT	At4g35720	Putative protein	A
19346_AT	At4g01630	Putative expansin At-EXP17	D
14643_S_AT	At2g03760	Putative steroid sulfotransferase	D
14517_AT	At2g41800	Putative protein	D
15178_S_AT	At4g14130	XTR7	E
15098_S_AT	At4g35770	Senescence-associated protein sen1	E
16078_AT	At3g16500	Phytochrome-associated protein 1 (PAP1)	E
17977_AT	At4g01680	MYB55	E
14062_AT	At2g47780	Putative protein	F

between BR and auxin occur in elongating tissues and cells in dicots (Yopp et al., 1981; Cohen and Meudt, 1983; Katsumi 1985; Sala and Sala, 1985) and monocots (Yopp et al., 1981; Takeno and Pharis, 1982), including bending responses. Tissue elongation or cell expansion is considered an important response for understanding interactions between auxins and BR, but the molecular mechanisms by which they interact and regulate plant tissue elongation are poorly understood. Xu et al. (1995) reported that the Arabidopsis *TCH4* gene, which encodes a xyloglucan endotransglycosylase, was induced quickly by IAA but rather slowly by BL. We have also reported that potential cell wall-related genes (*TCH4*, *AtExp8*, and *KCS1*) are induced quickly by IAA but slowly by BL, and that BL regulates a number of cell wall-related genes (Goda et al., 2002).

Here, we identified at least 100 genes potentially involved in cell wall organization as IAA or BL regulated. These genes include those encoding cell


A
Expression Level (Signal Log Ratio)
2.0
1.0
0.5
0.0
-0.5
-1.0
-2.0
IAA 15min
IAA 30min
IAA 3h
IAA 12h
IAA 24h
BL 15min
BL 30min
BL 3h
BL 12h
BL 24h
A B C D E F G H I J K L M N
Classification in Tables
B
Number of IAA regulated genes
150
100
50
0
early-Down
late-Down
early-Up
late-Up
C
Number of BL regulated genes
300
250
200
150
100
50
0
early-Down
late-Down
early-Up
late-Up
D
Cell Elongation
Unknown
Cellular Metabolism
P450
Plant Hormone
SAUR, GH3, and IAA gene families
Signal Transduction
Transcription
Transpoter / Channel
Others
IAA- or BL- regulated genes
E
Cell Elongation
Unknown
Cellular Metabolism
Others
Transpoter / Channel
P450
Plant Hormone
Transcription
Signal Transduction
SAUR, GH3, and IAA gene families
Genes regulated by both IAA and BL

wall synthesis enzymes, cell wall modifying agents, cell wall component proteins, and wall rigidification and wax-related proteins and included all the functional subcategories necessary for the completion of cell wall organization. This revealed the global manner by which these hormones regulate cell wall-related genes. We observed overlap and divergence of IAA and BL in regulating the genes involved in cell wall organization and cell elongation. Genes in this category are mainly early BL-up-regulated genes (Figs. 4A, green lines, and 1C) and not early IAA-up-regulated genes (Figs. 4A, red lines, and 1B). The majority of BL-regulated genes were up-regulated, and only five were down-regulated, whereas the numbers of IAA genes up- and down-regulated were comparable (Fig. 4A). Some members (e.g. β-1,3-glucanase, chitinase, peroxidase, and Leu-rich repeat proteins with or without extensin region) in this category are annotated as pathogen-related or disease resistance-related genes in a database based on their research history. However, we classified them as cell wall-related genes since recent studies have revealed that these genes are involved in multiple biological processes (Baumberger et al., 2001; Hrmova and Fincher, 2001; Passarinho and deVries, 2002; Yoshida et al., 2003). Since many genes await characterization to understand cell wall biogenesis and cell expansion, the genes listed and classified in Tables I to III should prove useful for identifying novel cell wall-related genes and further understanding cell wall biogenesis.

The majority (81%) of this gene category was composed of cell wall modifying agents, including xyloglucan endotransglucosylase/hydrolases (XTH), glucanase, polygalacturonase, pectin esterase, expansin, extensin, and chitinase. The most abundant cross-linking glycan in the primary cell wall of dicots is xyloglucan, which is thought to play an essential role in cell wall loosening and cell expansion. There are 33 *XTH* genes in the Arabidopsis genome (for review, see Rose et al., 2002), and they are classified into three major phylogenetic groups (Yokoyama and Nishitani, 2001). We found that 11 of them were regulated by IAA or BL. Interestingly, most of them belonged to group 1 or group 2. By contrast, only one exception (*AtXHT33*) belonged to group 3, although all the members of group 3 were represented on the array. This trend was reproduced in our whole-genome array experiments (H. Goda and Y. Shimada, unpublished data). Interestingly, members of groups 1 and 2 mediate transglucosylation between xyloglucans (Nishitani and Tominaga, 1992; Xu et al. 1996), while members of group 3 catalyze xyloglucan endohydrolysis (Fanutti et al., 1993). The responses of *XTH* genes in our data are consistent with previous studies of IAA regulation (Xu et al., 1995, 1996; Sawa et al., 2002) and BR regulation (Xu et al., 1995, 1996; Goda et al., 2002), except that some minor responses differed from those in a report by Yokoyama and Nishitani (2001). Although the reason is unclear at present, one possible explanation is that minor responses may depend on the experimental conditions, such as growth or hormone-treatment conditions.

Analysis of the Prompter Regions of IAA-Responsive and BL-Responsive Genes

Auxin response elements (AuxREs), which consist of a TGTCTC sequence and an adjacent or overlapping coupling element, were defined based on the auxin-responsive promoter of the soybean *GH3* gene (Liu et al., 1994; Ulmasov et al., 1995). Gain-of-function experiments with minimal promoter-*GUS* (β-glucuronidase) reporter genes have shown that a single copy of an AuxRE is sufficient to confer auxin responsiveness to reporter genes (Ulmasov et al., 1995). DR5, an artificial AuxRE containing the TGTCTC element, has increased auxin responsiveness (Ulmasov et al., 1997). The *GUS* reporter gene fused to a minimal cauliflower mosaic virus 35S promoter and the DR5 AuxRE has been used widely as a marker to monitor the distribution of endogenous IAA, as it has been suggested that the resulting GUS activity coincides with this distribution (Sabatini et al., 1999; Casimiro et al., 2001).

As we found that a number of the early auxin-inducible genes are induced in response to BL treatment, we tested the frequency of BL-inducible genes possessing the TGTCTC element in the 5′-flanking region. The 8,300 genes represented on the Arabidopsis Genome Array corresponded to 7,388 independent loci in the Arabidopsis genome. The numbers of IAA- and BL-regulated genes containing the TGTCTC element or its inverse (GAGACA) were counted and are given as a proportion of the 7,388 genes (Fig. 5). At least one TGTCTC element exists 5′

Figure 1. Gene expression patterns in response to BL and IAA treatment. Seven-day-old WT seedlings were treated with IAA, or *det2* seedlings were treated with BL. Then transcript abundance was analyzed using an Affymetrix GeneChip representing about 8,300 Arabidopsis genes. A, The expression of 637 BL- or IAA-inducible genes (listed in Tables I–III). Colors (red to blue, defined to the left of the column) represent the magnitude of induction in SLR values relative to mock-treated samples. Genes were clustered hierarchically using GeneSpring and grouped into groups A to N (at the bottom of the column). The trees at the top (in blue lines) indicate similarity in the gene expression patterns. The horizontal color bars (at the bottom) represent the classification used in the tables, namely IAA-regulated genes (red), BL-regulated genes (green), and genes regulated by both BL and IAA (yellow). B to E, The frequencies of IAA- and BL-regulated genes. The IAA- and BL-regulated genes are classified into 10 functional categories (indicated in D and E) based on their established or putative functions. The genes induced more than 2-fold within 3 h of hormone treatment are defined as early inducible genes, and those induced between 12 h and 24 h are defined as late inducible genes. The numbers of IAA- (B) or BL-regulated genes (C) are shown. The frequencies of genes regulated by IAA or BL (D) or both (E) are shown.

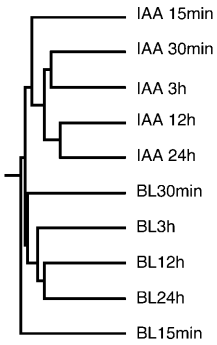


Figure 2. Relationships between the BL and IAA treatments. The dendrogram was calculated by hierarchical clustering using data on the expression of 637 BL- or IAA-regulated genes (listed in Tables I–III). The dendrogram represents the similarity of the gene expression profiles with the BL and IAA treatments at each time point.

upstream from the start codon within 1,000 bp of 1,817 genes (25%) or within 500 bp of 1,071 genes (14%). Similarly, the inverse element, GAGACA, exists within 1,000 bp of 1,640 genes (22%) or within 500 bp of 863 genes (12%). Surprisingly, the TGTCTC element was most frequent for genes regulated by both IAA and BL, rather than in genes up-regulated specifically by IAA (Fig. 5). The frequency of genes with multiple TGTCTC elements was also highest in these genes. This is consistent with our recent finding that BL treatment induces the DR5-*GUS* gene in Arabidopsis (Nakamura et al., 2003a). We also demonstrated that the early auxin-inducible genes *IAA3*, *GH3-2/BRU6*, *SAUR-AC1* (Goda et al., 2002; Nakamura et al., 2003b), *IAA5*, and *IAA19* (Nakamura et al., 2003a) are induced with similar kinetics to the DR5-*GUS* gene in Arabidopsis, namely they are quickly and transiently induced by IAA and gradually and continuously induced by BL. Furthermore, BL induces *SAUR-AC1* (Nakamura et al., 2003b), *IAA5*, *IAA19*, and DR5-*GUS* (Nakamura et al., 2003a) in a manner independent of the endogenous auxin levels. Consequently, we speculate that genes up-regulated by both BL and IAA are regulated by a common cis-regulatory element, which includes TGTCTC. Interestingly, the frequency of genes having the TGTCTC element was lower in genes down-regulated by both BL and IAA, although as there were only seven such genes, this result could be due to an artifact. However, this trend was also observed in early BL-down-regulated genes, late BL-down-regulated genes, and late IAA-down-regulated genes (data not shown). Furthermore, the inverse element (CGCACA)

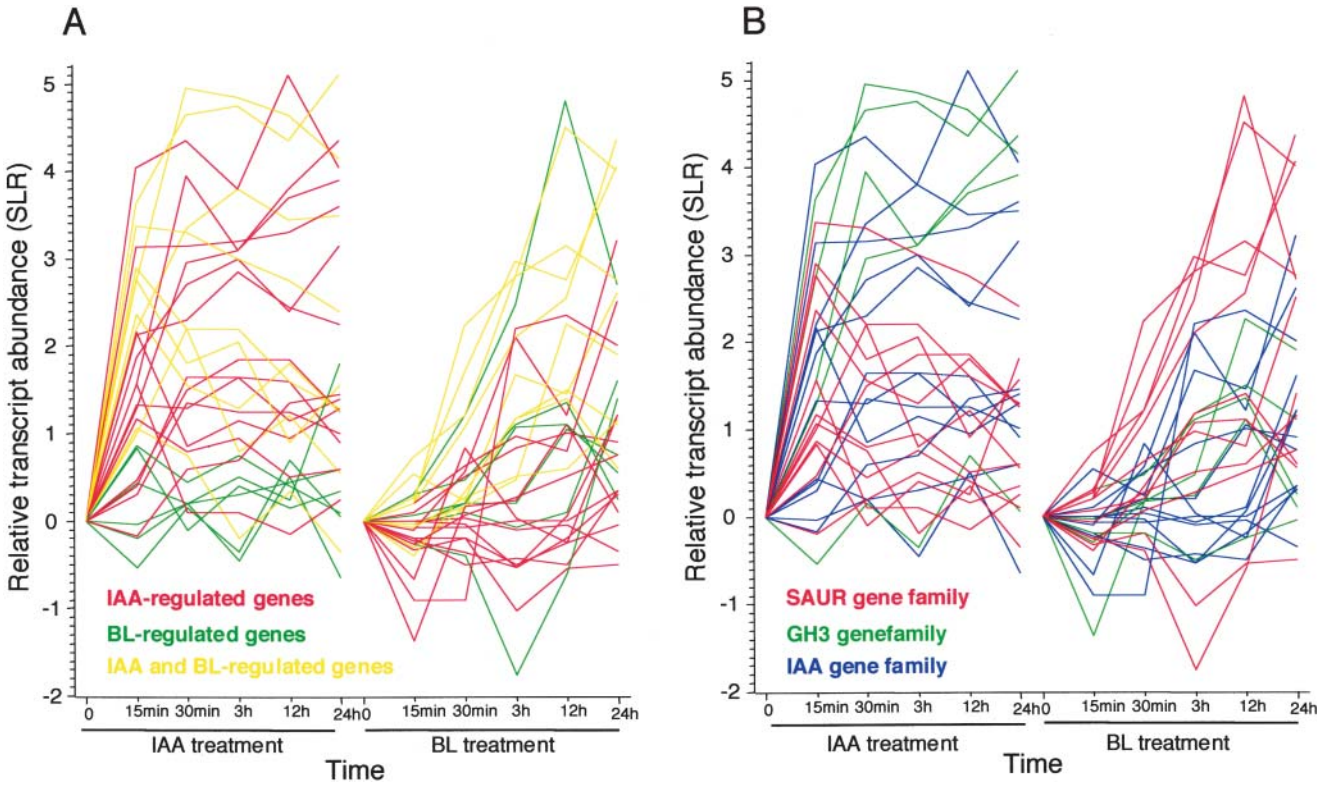


Figure 3. Induction kinetics of *SAUR*, *GH3*, and *IAA* genes. Transcript abundance of IAA- or BL-regulated *SAUR*, *GH3*, and *IAA* genes relative to mock-treated samples is given in SLR values. The data are given as the means of three or two independent hormone-treated plant samples. A, Colored according to hormone inducibility: regulated by IAA (red), BL (green), or both (yellow). Some genes that were induced more than 2-fold in both treatments were classified as being regulated by one hormone if the induction with the other hormone was not significant based on the results of the MAS (version 5) analysis. B, *SAUR*, *GH3*, and *AUX/IAA* family genes are shown in red, yellow, and blue, respectively. The value at 0 h is a theoretical value (0) and is not based on experimental results.

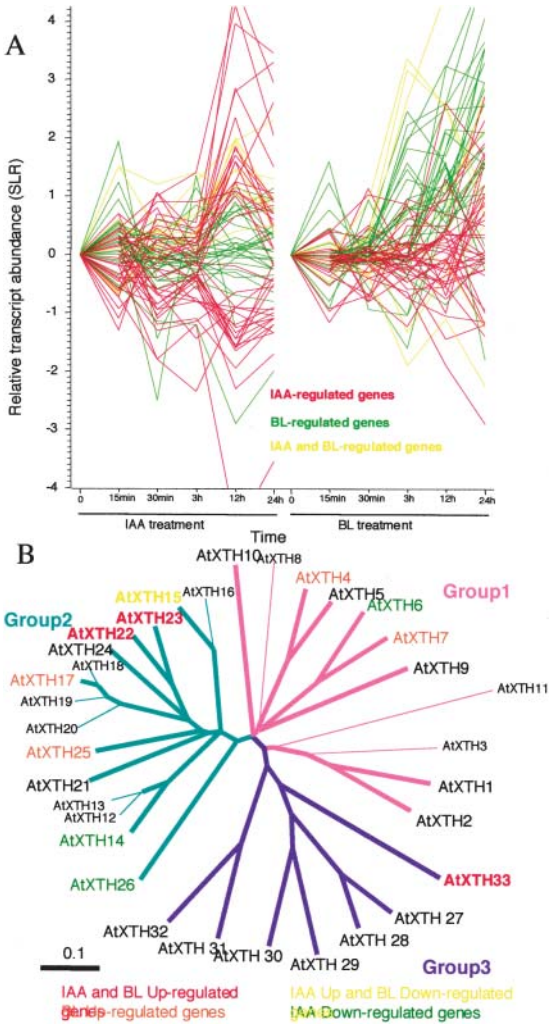


Figure 4. Regulation of genes involved in cell expansion or cell wall organization. A, The induction kinetics of genes involved in cell expansion or cell wall organization. Transcript abundance of IAA- or BL-regulated genes in this category relative to mock-treated samples is shown as SLR values. Genes regulated by IAA, BL, or both are shown in red, green, or yellow, respectively. The data are shown as the means of three or two independent hormone-treatment experiments. Some genes induced more than 2-fold by both hormone treatments were classified as specifically regulated by one hormone if induction with the other hormone was not significant based on the results of the MAS (version 5) analysis. The value at 0 h is a theoretical value (0) and is not based on experimental results. B, Regulation of XTH genes by IAA or BL. A phylogenetic tree of the Arabidopsis *XTH* gene family was generated using ClustalW and TreeViewPPC software based on the deduced amino acid sequences of all 33 Arabidopsis *XTH* genes. Genes from groups 1, 2, and 3 are shown with pink, green, and violet lines, respectively. The color coding of the letters is as follows: genes up-regulated by both IAA and BL (red letters); IAA-up- and BL-down-regulated genes (yellow); IAA-down-regulated genes (green); BL-up-regulated genes (orange); and genes not regulated by either IAA or BL (black). Genes not represented on the Affymetrix Arabidopsis Genome Array are shown in small letters.

was not enriched in genes up-regulated by both IAA and BL but was enriched in genes down-regulated by both (Fig. 5), even though it is generally believed that the inverse element has the same function as the orthodromic element. This trend was also observed in early BL-down-regulated genes (data not shown). These findings will be useful for future studies to understand the roles of TGTCTC and the inverse element in BR- and auxin-regulated gene expression, as well as to identify novel cis-regulatory elements that are specific to BL or IAA regulation and to elements involved in down-regulation.

Other Interactions between BR and Auxin

We found the following responses, which may be important to further understanding auxin/BR interactions. Three genes potentially involved in signal transduction pathway were newly identified here as being induced by both BL and IAA: a homolog (At2g30040) of the brassinosteroid-insensitive 2 kinase gene (*BIN2*; Choe et al., 2002; Li and Nam, 2002; Perez-Perez et al., 2002), *At4g27280* encoding calcineurin B-like protein, and *At1g74650* encoding a putative transcription factor (Myb-like). We previously reported

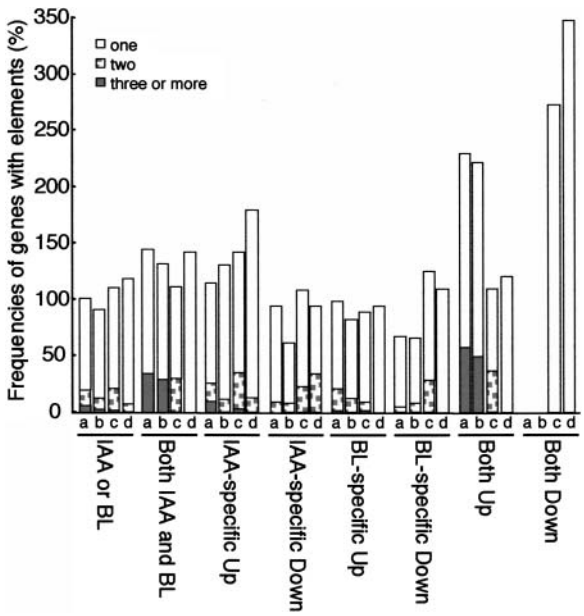


Figure 5. Frequencies of genes with TGTCTC or GAGACA elements in the 5′-flanking region of IAA- or BL-regulated genes. The numbers of genes containing TGTCTC or its inverse element (GAGACA) in the 5′-flanking region (up to −500 or −1,000 bp) were calculated using GeneSpring. The frequencies of genes with these elements are given as a proportion of the 7,388 independent loci represented in the Arabidopsis Genome Array (about 8,300 genes corresponding to 7,388 independent loci). a, TGTCTC (−1,000 bp); b, TGTCTC (−500 bp); c, GAGACA (−1,000 bp); d, GAGACA (−500 bp). The shading in each bar indicates the ratios of the genes containing one, two, or three and more elements.

that *PIN7*, a homolog of the *PIN1* and *PIN2* genes for putative auxin-efflux carrier proteins (Galweiler et al., 1998; Muller et al., 1998), was repressed by BL treatment (Goda et al., 2002). This response was confirmed here. BRI1 is a critical component of the BR receptor (Wang et al., 2001). Three genes encode BRI1-like proteins in Arabidopsis: *BRL1*, *BRL2*, and *BRL3*. *BRL1* and *BRL3* are reported to bind BL (Yin et al., 2002b). In this study, *BRL3* (At3g13380) was up-regulated in response to IAA treatment later on. Conversely, we observed that *BAS1/CYP72B1*, which encodes an enzyme that inactivates BRs (Neff et al., 1999), was increased by IAA treatment later on. These results suggest that auxin regulates BR signaling and catabolism.

As described above, P450 genes constituted the largest group of early BL-down-regulated genes (Fig. 1C), while relatively few P450 genes were IAA-regulated genes, perhaps because a number of P450 genes are involved in BR biosynthesis and catabolism (Fujioka and Yokota, 2003). Conversely, none of the genes involved in auxin metabolism were identified here as IAA regulated. *BAK1* encodes the Leu-rich repeat receptor-like kinase belonging to the Leu-rich repeat receptor kinase II and X family (http://plantsp.sdsc.edu/plantsp/family/class). Overexpression of the *BAK1* gene leads to a phenotype reminiscent of the *BRI1*-overexpression transgenic plant, and BAK1 protein interacts with BRI1 in vivo and in vitro (Li et al., 2002; Nam and Li, 2002). We found that a *BAK1* homolog (At2g13790), the gene most closely related to *BAK1* in the Leu-rich repeat receptor-like kinase gene family of Arabidopsis, was induced by BL treatment at a later time point. It will be interesting to test whether the At2g13790 gene functions in the BR signaling.

Cross Talk with Other Plant Hormone Signaling

Earlier studies reported that IAA and BR exhibit cross talk with other plant hormones. In Arabidopsis, BL induced the *OPR1* (Goda et al., 2002) or *OPR3* (Müssig et al., 2000) genes encoding 12-oxophytodienoic acid 10,11-reductase involved in jasmonate biosynthesis (Biesgen and Weiler, 1999). BL also induced the GA 20-oxidase gene (*AtGA20ox1*; Bouquin et al., 2001). IAA treatment induced the 1-aminocyclopropane-1-carboxylate (ACC) synthase gene (*ACS*; Abel et al., 1995). These hormone cross talk responses observed previously in Arabidopsis were confirmed here. In addition, BL induction of *ACS* has been reported in mung bean (*Vigna radiata*; Yi et al., 1999). Auxin regulation of the *GA20ox* gene has been well studied in the pea (Van Huizen et al., 1997; Ngo et al., 2002; O'Neill and Ross, 2002). These responses found in other species were confirmed here in Arabidopsis for the first time, to our knowledge. Namely, BL induced *AtACS4*, and IAA induced *AtGA20ox1*. In addition, we found that BL induced *AtGA2ox8*, which encodes GA-inactivating enzyme (Schomburg et al., 2003). IAA repressed the cytokinin oxidase gene (*CKX4*), which encodes an enzyme that inactivates cytokinin (Bilyeu et al., 2001). These novel findings could be clues to unravel complex phytohormone cross talk and plant signaling networks.

CONCLUSIONS

To our knowledge, this is the first comprehensive expression profiling study of either auxin or BR over time. In addition, this is, to our knowledge, the first report to investigate the relationship between the actions of auxin and BR using a comprehensive expression profiling approach. The time course experiment revealed overlap and divergence between the actions of these two hormones. We identified 637 genes regulated by IAA or BL. Of these, 48 were regulated by both IAA and BL. Most BR actions are mediated by the induction of genes that are independent of the auxin response. The *SAUR*, *GH3*, and *IAA* gene families were the largest group of genes regulated by both IAA and BL. A number of the early auxin-inducible genes are not specifically regulated by auxin, but are regulated by these two hormones in common. Conversely, this study revealed true auxin-specific and BR-specific genes. This classification of genes is important for understanding the functional divergence and interaction of auxin and BR. A previously reported TGTCTC element in AuxRE was not enriched in genes specifically regulated by IAA but was enriched in genes up-regulated by both BL and IAA. This observation is consistent with our previous findings that a synthetic AuxRE, *DR5*, responded to both IAA and BL with kinetics similar to those of *IAA* or *SAUR* genes, independent of the endogenous auxin level (Nakamura et al., 2003a, 2003b). Therefore, the DR5-*GUS* reporter system is not specific to auxin action, but is an important marker for studying the BR/auxin interaction. About 30% of IAA- or BL-regulated genes were classified in an unknown category. A classification based on expression analysis will be useful for elucidating the functions of these genes and should provide insight into the activities of auxin and BR. For example, since all known BR-biosynthetic genes were classified in group E, this group may include genes that are important for BR biosynthesis and action.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis ecotype Colombia (Col-0) was used as the WT in this study. The Arabidopsis mutant *det2-1* (Chory et al., 1991) was used as a BR-deficient mutant. Seedlings were grown for 7 d at 22°C under continuous light in half-strength Murashige and Skoog (1962) liquid medium (Gibco BRL, Cleveland) supplemented with 1.5% (w/v) sucrose. The seedlings were then treated with 1 μM IAA or 10 nM BL or mock treated with dimethyl sulfoxide (final concentration 0.1%). Then, they were immediately frozen in liquid nitrogen and stored at −80°C until RNA isolation.

DNA Microarray Analysis

The DNA microarray analysis was performed essentially as described previously (Goda et al., 2002). Total RNA was isolated from seedlings using the acid-guanidinium-phenol-chloroform method (Sambrook et al., 1989) and converted into double-stranded cDNA using a Super Script Choice cDNA synthesis kit (GIBCO BRL) with an oligo$(dT)_{24}$ primer containing a T7 polymerase promoter site at its 3′ end (Amersham Pharmacia Biotech, Uppsala). Biotin-labeled cRNA was generated from the double-stranded cDNA using a BioArray HighYield RNA transcript labeling kit (Enzo Biochem, Farmingdale, NY) and was then purified using an RNeasy RNA purification kit (Qiagen USA, Valencia, CA). Each cRNA sample (20 μg) was fragmented and hybridized with the Arabidopsis Genome Array (Affymetrix, Santa Clara, CA) for 16 h at 45°C with rotation at 60 rpm. Each array was then washed and detected by consecutive exposure to phycoerythrin-streptavidin (Molecular Probes, Eugene, OR), biotinylated antibodies to streptavidin (Vector Laboratories, Burlingame, CA), and phycoerythrin-streptavidin, after which each array was washed again with a nonstringent wash buffer. All washing and staining procedures were performed with a Fluidics Station 400 (Affymetrix). The array was scanned using a confocal microscope scanner (HP Genome Array Scanner; Affymetrix) at a wavelength of 570 nm. To achieve a higher signal dynamic range, we scanned each chip before and after signal amplification using an anti-streptavidin antibody. Each chip was normalized relative to the sum of the signal values, and then the control and IAA- or BL-treated samples were compared at each time point using the GeneChip software MAS version 5 (Affymetrix). Genes that were assigned as up- or down-regulated on the basis of more than a 2-fold difference in their signal values and that were assigned as Increase or Decrease on the basis of their Change values were extracted. Furthermore, genes with Absent for the Detection value in the baseline data and Decrease for the Change value were excluded from the list. Similarly, genes with Absent for the Detection value in the experimental data and Increase for the Change value were also excluded from the list. To ensure the reproducibility of the results, we performed three (15 min for IAA treatment; 15 min, 30 min, and 3 h for BL treatment) or two (for other time points) independent hormone-treatment experiments with different plant samples. Genes that showed reproducible responses in all experiments were classified as genes regulated by IAA or BL at each time point of the treatments (Tables I–III). This threshold seems to be more stringent for false-positive genes than the conventional threshold based solely on a statistical analysis (Welch's t test, at a significance level of $P < 0.05$) of signal values in independent experiments, as described previously (Goda et al., 2002).

The SLR was imported into GeneSpring software (version 4; Silicon Genetics, Redwood, CA), and the genes were clustered hierarchically based on the angular separation of the expression vectors for each gene. Elements of the 5′-flanking regions were also analyzed using GeneSpring software.

Annotations and Database Analysis

Since the locus assignments and annotations of genes provided by Affymetrix contain errors, we used information provided by The Arabidopsis Information Resource (TAIR; files available at ftp://tairpub:tairpub@ftp.arabidopsis.org/home/tair/Microarrays/Affymetrix/). This information was produced by BLASTing the array element sequences downloaded from the Affymetrix Web site (http://www.affymetrix.com) against the Arabidopsis bacterial artificial chromosomes, chloroplast, and mitochondria genomes from The Institute for Genomic Research using BLASTN with an E value cutoff of $1e^{-6}$. The TAIR annotations were further revised manually using BLAST searches of the GenBank/EMBL/DNA Data Bank of Japan (DDBJ) databases, as well as reference searches on the ISI Web of Science.

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