



Transcriptome analysis reveals potential mechanisms for inhibition of intumescence development by UV radiation in tomato



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ABSTRACT

Intumescence is a physiological disorder characterized by epidermal cell hypertrophy in many plant species, including tomato. Although previous studies have been focused on identifying the environmental conditions associated with intumescence development, fundamental molecular responses of plants during the development of the disorder are unknown. Our recent work demonstrates that intumescences can be induced by blocking plant exposure to ultraviolet (UV) radiation. To understand how UV influences intumescence development at the molecular level, we grew an intumescence-sensitive tomato cultivar, Maxifort, under environmental conditions either with UV or blocked-UV. Extensive intumescences were induced in leaves grown under the blocked-UV condition, and a comparison of the gene expression profiles of leaf tissues with and without intumescences indicated that there were 1604 genes differentially expressed. The genes were involved in several important cellular processing groups, including hormone response, DNA synthesis and repair, metabolic pathways and cell wall biosynthesis. Furthermore, most of the photosynthesis-associated genes were uniformly repressed in the leaves with intumescences. Ethylene biosynthesis and its downstream signal transduction pathway were particularly more active in leaves with intumescences than in those without intumescences, suggesting that ethylene signaling may play a role in occurrence of intumescence. We further analyzed the interaction between UV treatment and intumescence-related genes to determine how UV inhibits the development of intumescences and identified a number of genes that could be induced by UV treatment but suppressed in the leaves with intumescences. Among them, an important gene, 3-beta hydroxysteroid dehydrogenase (*3β-HSD*), might play a key role in UV inhibition of intumescence development. Taken together, the results of this study provide insights into the mechanisms regulating the development of intumescences at the molecular level in tomato.

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1. Introduction

Intumescences are a physiological disorder characterized by hypertrophy of the upper or lower epidermal and palisade parenchyma cells of leaf tissue (Lang et al., 1983; Craver et al., 2014b). It develops sporadically on the foliage of many plant species, including some varieties of tomato (*Solanum lycopersicum* L.) and sweet potato (*Ipomoea batatas* L. Lam.) (Wetzstein and Frett, 1984; Craver et al., 2014b). The lesions are described as abnormal, translucent outgrowths on the leaf surface with a gall or wart-like appearance (Wetzstein and Frett, 1984; Morrow and Tibbitts,

1988). Extensive intumescence development, or any extensive leaf blotching, results in impaired photosynthesis (Roloff et al., 2004; Pinkard et al., 2006), and the cellular abnormalities associated with intumescence ultimately cause cell collapse and tissue senescence, having a substantial negative impact on both the economic and aesthetic value of affected plants.

Intumescences mostly develop on plants being produced in controlled environmental conditions (Lang and Tibbitts, 1983; Wetzstein and Frett, 1984; Petite and Ormrod, 1986; Jaworski et al., 1988). Proposed causative factors include air contamination (Kirkham and Keeney, 1974; Lang and Tibbitts, 1983), phytohormones (White, 1951; Kirkham and Keeney, 1974; Lang et al., 1983; Petite and Ormrod, 1986; Morrow and Tibbitts, 1988), and light quality (Lang and Tibbitts, 1983; Morrow and Tibbitts, 1988). Ultraviolet (UV) radiation, in particular, has been found to be connected to the disorder, as intumescences occur predominantly

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in protected culture and many greenhouse-glazing materials block UV radiation wavelengths (100–400 nm). In addition, UV radiation has been shown to abate intumescence development on tomato (*Solanum lycopersicum* L. var. *hirsutum* and *S. lycopersicum* var. *esculentum* 'Oxheart') (Lang and Tibbitts, 1983) and *S. lycopersicum* 'Maxifort' Craver et al., 2014b; Williams et al., 2015), ornamental

sweet potato (*Ipomoea batatas* 'Ace of Spades') (Craver et al., 2014a), and *Cuphea* species (Jaworski et al., 1988). However, a mechanistic link between UV radiation and intumescence development has not been studied.

To unravel the mechanisms of intumescence development and crosstalk with UV radiation, we first compared the transcriptional

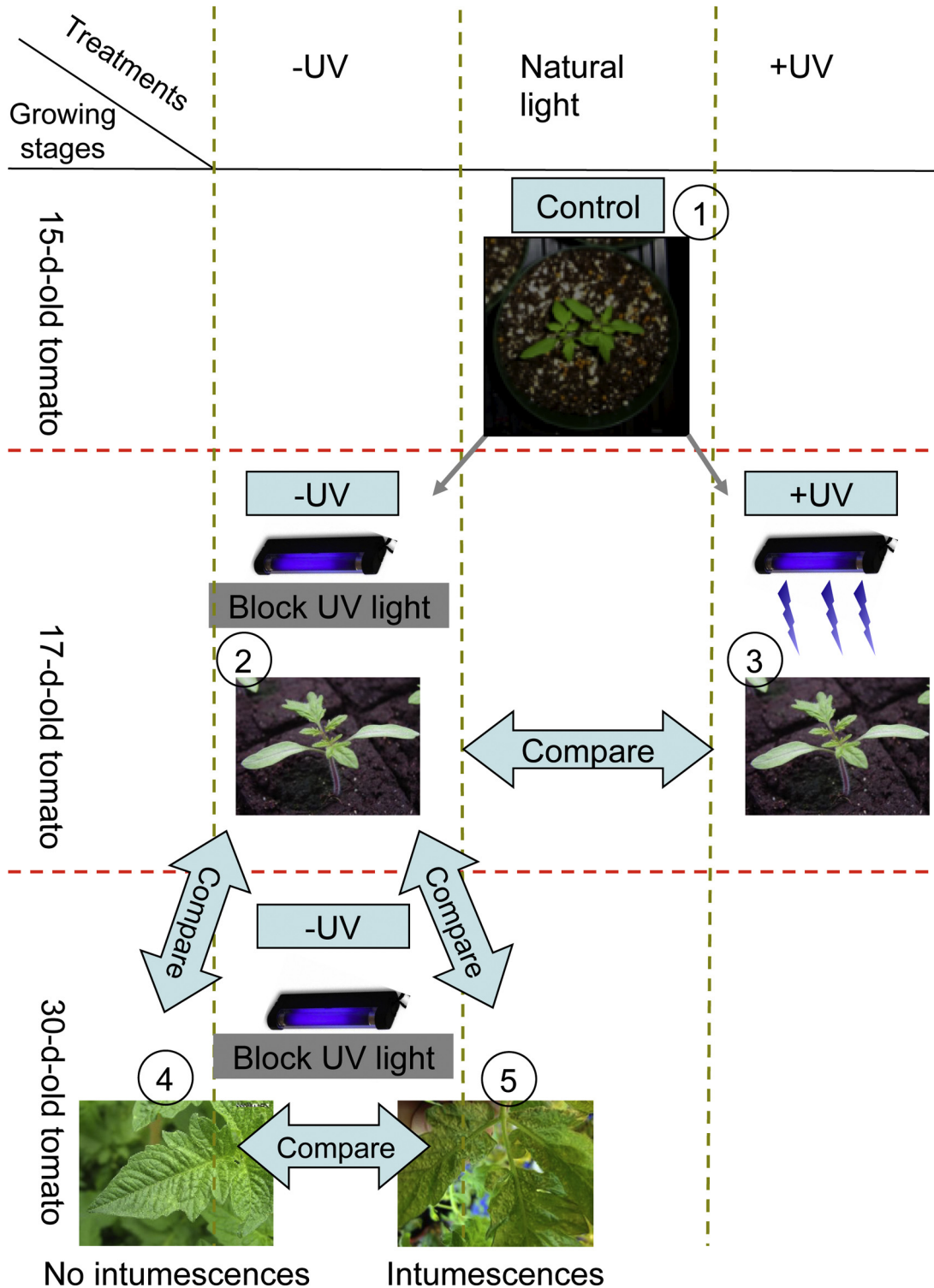


Fig. 1. Experimental design. 15-d-old tomato seedlings were treated with either UV or blocked-UV. After being treated for 2 days, the true leaves of 17-d-old grown under UV radiation and blocked-UV were sampled, respectively. The seedlings that were treated by blocked-UV were further grown under the blocked-UV condition for 13 more days to induce intumescences. The 30-d-old leaves that were grown under the blocked-UVB condition, either with or without intumescences, were sampled.

profiling of tomato leaf tissues with or without intumescence to identify the genes that are differentially regulated in the leaves with intumescent tissue. We further identified several important genes that were oppositely regulated between the healthy and intumescent leaves during leaf development and that have potential to be applied toward engineering or breeding intumescence resistant tomatoes after further functional studies. Finally, we identified a set of UV-regulated genes that might be involved in the development of intumescences. Our findings at the molecular level will facilitate understanding of the physiological changes of tomato during the development of intumescences.

2. Materials and methods

2.1. UV and blocked-UV environments

Two structures were built out of metal conduit arches attached to an expanded metal greenhouse bench in a glass-glazed greenhouse. The UV radiation source consisted of six UV transmitting fluorescent light tubes (UVB-313 lamps, Q-Lab) per structure that were mounted 88 cm above the bench. These lamps emitted wavelengths from 250 nm to 400 nm, but had the highest irradiance in the 290 to 340 nm range. Plastic light diffusers (Styrene Prismatic Clear; Plaskolite) with dimensions of 61 cm x 122 cm were installed 9 cm below the lights (79 cm above the bench surface). The area underneath the UV lamps and diffusers was separated in half with a vertical baffle of UV-block plastic (DuraGreen EM 3 Years OF D7/11; DuraGreen Marketing USA). One half of the structure was covered with UV-block plastic installed 4 cm below the diffuser and the other half of the structure was left open for plants to receive UV radiation.

The UV lamps were on 12 h each day, from 0700 HR to 1900 HR daily. On the date that 17-d-old tissue samples were collected, UV levels (250–400 nm) were $6.9 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the UV environment and $1.8 \pm 0.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the blocked-UV environment (N = 4; Field Scout 3414 Ultraviolet Light Meter, Spectrum Technologies).

2.2. Plant materials, growth conditions, and gene profiling comparison

We grew an intumescence-sensitive tomato cultivar, *S. lycopersicum* 'Maxifort', in a glass-glazed greenhouse from seed (Johnny's Selected Seeds). The temperature of the greenhouse was maintained within a range of 25–27 °C with a natural photoperiod from 5-Sept. to 5-Oct. in Manhattan, KS. After 15-d, we transferred the seedlings to one of two environments: UV or

blocked-UV for further growing. After another 2-d of growing, the 17-d-old true leaves under UV or blocked-UV conditions were harvested, respectively; no leaves had developed intumescences at this stage. After growing under blocked-UV conditions for 13 more days, at which time about half of the leaves under blocked-UV conditions had developed intumescences, 30-d-old true leaves with or without intumescences were harvested for gene profiling. The plants that were harvested at 17-d were not re-used at the next time point (Fig. 1).

The purpose of the gene profiling comparison was to 1) identify the mis-expressed genes in the intumescent leaves by comparing the gene expression profiles of 30-d-old leaves with or without intumescences; 2) identify the genes that may play essential roles in development from young, healthy to intumescent leaves by highlighting the oppositely regulated genes of the healthy or intumescent leaves during leaf development from 17- to 30-d-old leaf tissue; and 3) understand how UV influences intumescence by finding the overlapped genes, especially negatively correlated genes, that are affected by both intumescences and UV.

2.3. Transcriptional analysis

The expression analysis was accomplished in three biological replications. Total RNA was extracted from tomato leaves using the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The RNA quality of these samples was assessed by Agilent Bioanalyzer 2100 (Agilent Technologies). Total RNA (~300 ng) was processed for the microarray hybridization using the Affymetrix GeneChip 3' IVT Express Kit (Affymetrix). The resultant biotinylated copy RNA was fragmented and hybridized to the GeneChip Tomato Genome Array, which contains 10,209 tomato probe sets that interrogate more than 9200 tomato genes. The arrays were washed, stained, and scanned at the Integrated Genomics Facility at Kansas State University (<http://www.ksre.ksu.edu/igenomics/>). The data were imported into GeneSpring software (Agilent Technologies), and normalized with the 50th percentile shift. The unpaired *t*-test was used to calculate *P* values. Genes with *P* < 0.05 and 2-fold or greater change in expression were considered to be differentially expressed and gathered for further analysis. Annotations of differentially expressed genes were obtained by BLAST search comparison with the National Center for Biotechnology Information (database) by Blast2GO program (Conesa et al., 2005). Functional classifications were accomplished with MapMan software, a user-driven tool that displays a large data set as diagrams of metabolic pathways or other processes, and the gene ontology (GO) enrichment function

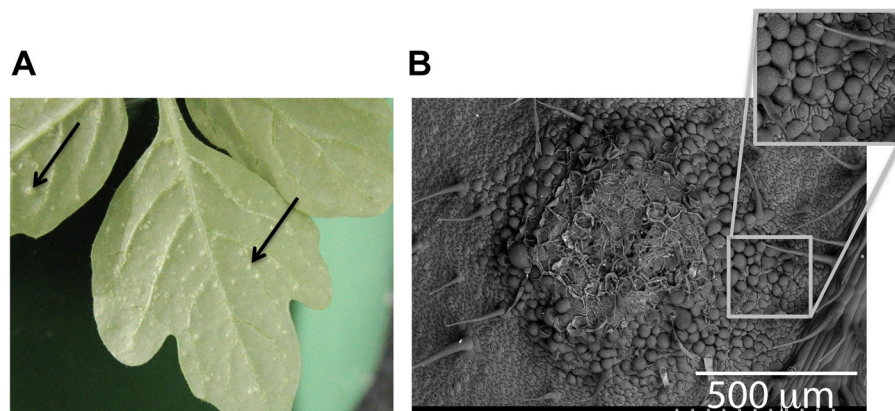


Fig. 2. Phenotype of the intumescent leaves. (A) abaxial side of the intumescent leaves. (B) SEM of the senesced region (Scale bar = 500 μm).

of Genespring (Thimm et al., 2004). The pathway analyses were conducted by the KEGG database (Kanehisa and Goto, 2000; Kanehisa et al., 2014).

2.4. qPCR verification of the microarray data

The quantitative real-time PCR (qPCR) analysis was conducted in three biological replications using the same RNA sample as the microarray analysis. The cDNA was synthesized using the Advantage RT kit (Clontech) according to the manufacturer's protocol. qPCR was then performed in the iCycler iQ™ Real-Time PCR Detection System (Bio-rad) using the iQ™ SYBR Green Supermix (Bio-rad) kit. The data obtained were normalized based on the expression of the housekeeping gene *SIPP2ACS* (Lovdal and Lillo, 2009). The primers for the qPCR experiments are listed in Supplemental Table 1.

2.5. Scanning electron microscopy (SEM) analysis

Intumescent leaf tissue was collected from 'Maxifort' tomato leaves and the abaxial surface was imaged with SEM. Small pieces of leaf (~75 mm²) were glued onto an SEM slide using a graphite emulsion. The slides were placed into the SEM (S-3500N Hitachi Science Systems Ltd., Hitachinaka, Japan) and were rapidly cooled using liquid nitrogen to fix the samples. Micrographs were taken under high vacuum using a backscatter detector (Robinson Detector ETP-USA/Electron Detectors Inc., Rocklin, CA).

3. Results

3.1. Phenotypic analysis of intumescent leaves

Intumescences developed on the abaxial surface of the tomato leaves on the plants grown under the blocked-UV condition. During intumescence development, epidermal cells enlarged and formed yellowish lesions (Fig. 2A), suggesting that the chloroplasts might be affected in the intumescent cells. We further evaluated affected areas by using SEM. As shown in Fig. 2B, epidermal and other affected mesophyll cells eventually collapsed. At the edge of senesced region, the shape of epidermal cells changed from irregular to round (Fig. 2B, enlarged panel), as the intumescent lesion continued to expand.

3.2. Significant portion of genes are involved in the development of intumescent leaves

To understand how intumescence affects global gene expression, we compared the expression levels and patterns of genes between 30-d-old intumescent and 30-d-old healthy leaves. The results suggested that 1604 genes were differentially expressed. Among them, 1028 genes were induced and 576 genes were repressed at least two-fold, with a *P* value below 0.05 (Supplemental Table 2 and 3; a view of the distribution of differentially expressed genes was provided by a volcano plot in Fig. 3A).

A scheme of the gene classification, which was conducted according to their known or predicted functions from MapMan, was presented (Fig. 3B). In addition, the GO enrichment results were listed in Supplemental Table S10 and S11 with a corrected *p*-value cutoff < 0.01. The function of a large portion of the differentially expressed genes remains unknown. The second large portion of genes associated with intumescence was related to primary metabolic pathways, such as amino acid metabolism and nucleotide metabolism. One hundred and ninety three genes related to metabolism were repressed, while only 97 were induced, indicating that the overall primary metabolic pathways were inhibited in the intumescent leaves. In addition, the data indicate

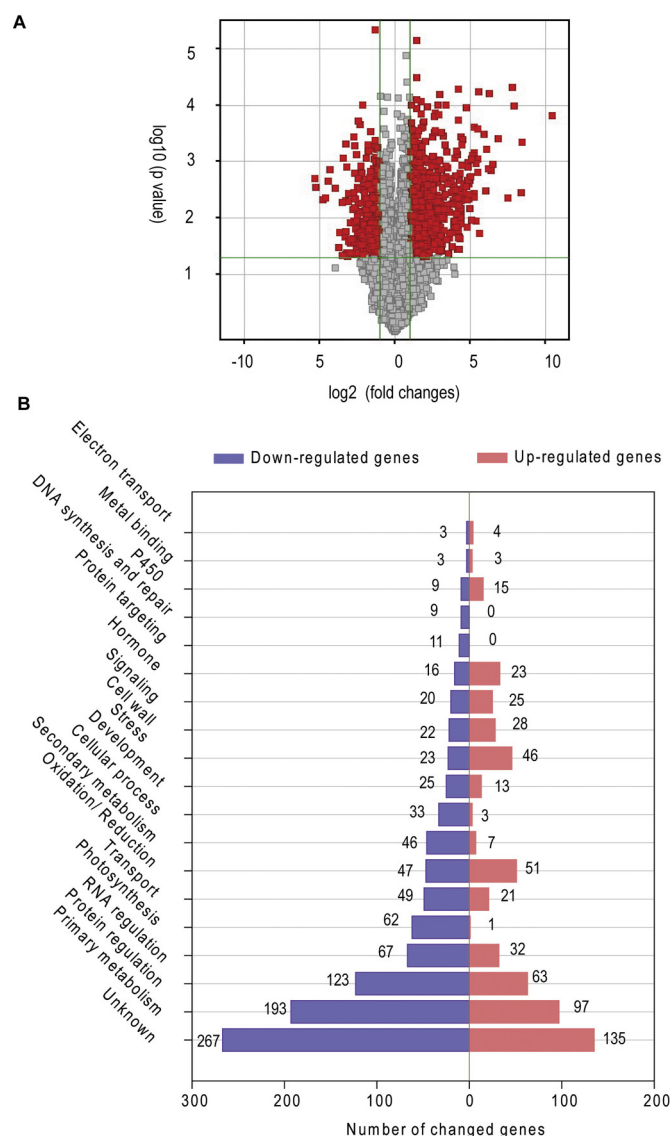


Fig. 3. Gene expression changes between the leaves with or without intumescences. In this data set, the leaves without intumescence are considered the control. (A) Volcano plot where log₂-transformed gene expression intensity ratios was plotted against the negative log₁₀-transformed *P* value from a Student's *t*-test. Genes with statistically different expression (*p* < 0.05) and fold changes above 2 were considered to be up-regulated genes. Genes with statistically different expression and fold changes below -2 were considered to be down-regulated genes. The changed genes are shown in red, and unchanged genes are shown in gray. (B) The differently regulated genes between intumescent and healthy leaves were shown in different functional groups. In most cases, the putative functional categories were assigned according to sequence homologies with previously studied genes from tomato or other species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that the secondary metabolisms were also severely inhibited in the intumescent leaves (Fig. 3B). The biotic and abiotic stress-related genes are one of a few groups in which the number of up-regulated genes (46) is higher than that of down-regulated genes (23; Fig. 3B).

3.3. Intumescent leaves show disorder in several important biological processes

We visualized the data to facilitate the identification of patterns of transcriptional change in various pathways using MapMan

(Thimm et al., 2004). We highlighted the changes of transcripts associated with photosynthesis, DNA repair and synthesis, oxidase, and flavonoid synthesis because the genes in the pathways showed uniform regulation patterns (Fig. 4). The photosynthetic- and photorespiration-associated genes covered in this tomato Genchips were predominantly repressed in the intumescent leaves (Fig. 4A). Additionally, all the DNA repair and synthesis associated

genes were also uniformly repressed in the intumescent leaves (Fig. 4B). In contrast, the oxidase genes were uniformly up-regulated in the intumescent leaves (Fig. 4C), while the flavonoids, including chalcones, isoflavonoids, and flavonols, synthesis related genes were uniformly down-regulated in the intumescent leaves (Fig. 4D).

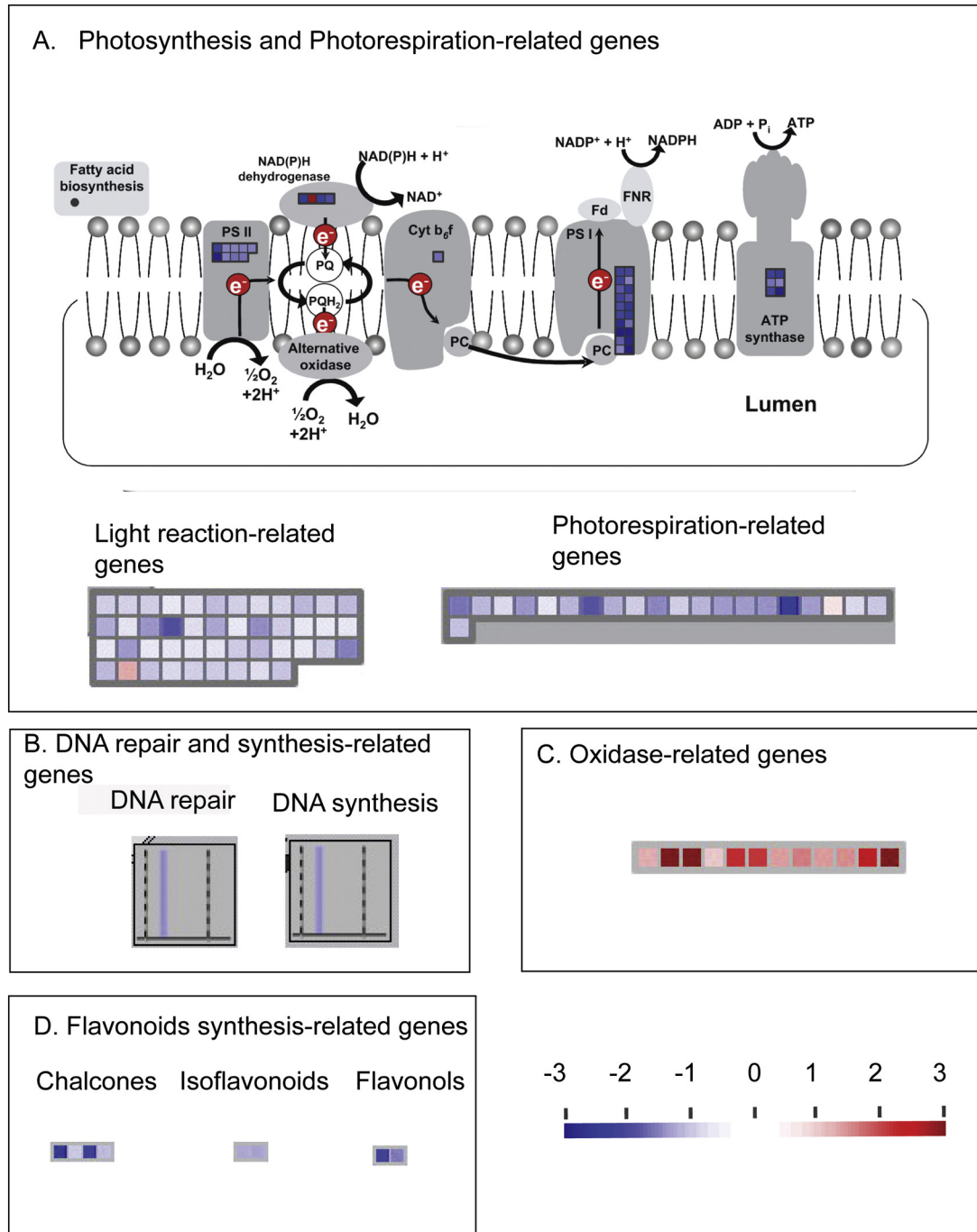


Fig. 4. The differentially expressed genes between intumescent and healthy leaves are associated with several important biological processes. (A) Most of the photosynthesis and photorespiration-related genes showed lower expression in intumescent leaves compared to healthy leaves. (B) In the intumescent leaves, the DNA repair and DNA synthesis-related genes showed lower expression compared to healthy leaves. (C) The oxidase-related genes showed higher expression in the intumescent leaves. (D) Flavonoids synthesis-related genes showed lower expression in the intumescent leaves. Red and blue represent increase and decrease, respectively. Here, each gene that was assigned to a process was represented by a single colored box. The view was constructed using MapMan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Ethylene synthesis and signaling are more active in the intumescent leaves

We closely examined the genes associated with plant hormone synthesis and signaling and found that the ethylene synthesis and

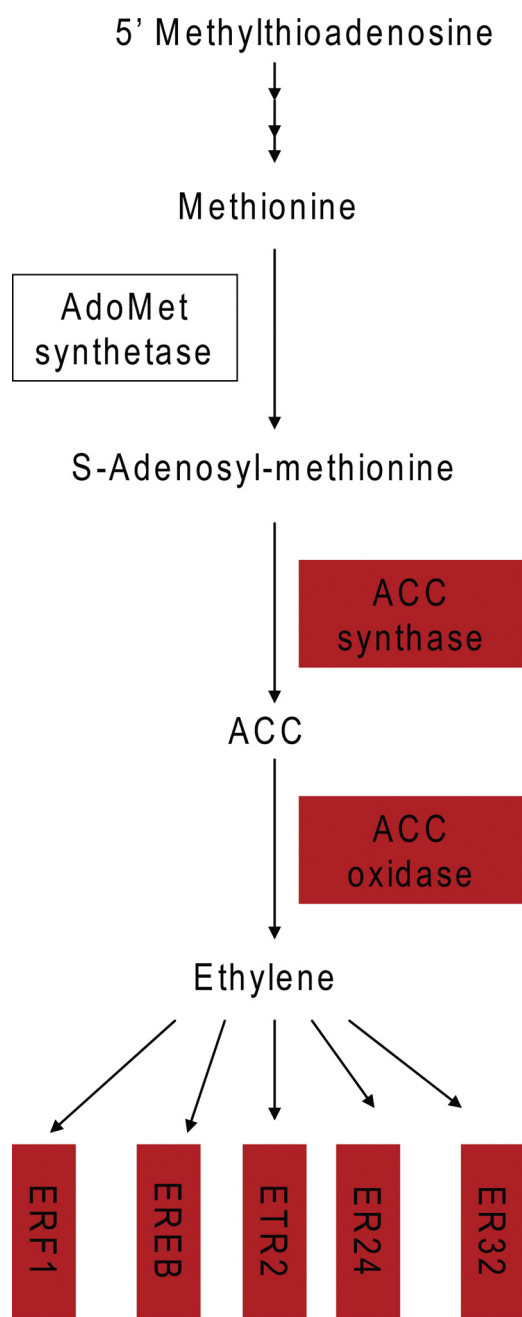


Fig. 5. Regulation of ethylene biosynthesis and signal transduction in the intumescent leaves as compared with healthy leaves. ACC synthases, including, ACS2 and ACS7 were up-regulated in intumescent leaves. ACC oxidases, including (ACO1, ACO4, and ACO6) were up-regulated in intumescent leaves. The genes related to ethylene downstream signal transduction including: ERF1 (ethylene response factor 1), EREB (ethylene responsive element binding protein), ETR2 (ethylene receptor 2), ER24 (ethylene-responsive transcriptional coactivator 24), ER32 (ethylene-responsive transcriptional coactivator 32) were up-regulated in intumescent leaves. Red color indicates up-regulated levels in the intumescent leaves. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

signaling pathway encountered dramatic changes in the intumescent leaves. Genes associated with ethylene synthesis and signaling were overwhelmingly up-regulated in the intumescent leaves as compared with healthy leaves. For example, ACC synthases (including ACS2 and ACS7), and ACC oxidases (including ACO1, ACO4 and ACO6), were up-regulated in intumescent leaves. Further, the genes related to ethylene downstream signal transduction, such as ERF1 (ethylene response factor 1), EREB (ethylene responsive element binding protein), ETR2 (ethylene receptor 2), ER24 (ethylene-responsive transcriptional coactivator 24) and ER32 (ethylene-responsive transcriptional coactivator 32), were also up-regulated in intumescent leaves (Fig. 5). The transcriptional profiling data showed that the ethylene pathway is stimulated in the intumescent cells, suggesting that it may play a pivotal role in the development of intumescences.

3.5. Intumescent and healthy leaves showed different gene regulations during development

Although the symptoms of intumescence were dramatically induced when the plants were grown under blocked-UV conditions, some leaves remained healthy on the same plants. This observation led us to investigate the differential regulation of genes between the healthy and intumescent leaves. Thus, we compared the transcriptional profiling of the 30-d-old healthy and intumescent leaves with 17-d-old healthy leaves to identify the genes that showed different regulation during leaf development. All the plants sampled were grown under the blocked-UV condition. As shown in Fig. 6A, the 30-d-old intumescent leaves showed 2232 differentially expressed genes, while the healthy leaves showed less than half that number—1005 differentially expressed genes, as compared with the 17-day-old leaves (Supplemental Table 6, 7, 8 and 9). There were 641 genes differentially expressed in both intumescent and healthy leaves during leaf development. Among them, 622 genes showed positive correlation; however, only 19 genes showed an opposite regulation pattern (Fig. 6B and C). This result indicates that the 19 genes might play key roles in determining whether the leaves would become intumescent or remain healthy. The 19 genes and their putative functions are listed in Table 1.

3.6. UV regulates a set of genes involved in the development of intumescence

Our previous research has shown that intumescence can be induced by growing plants under the condition of blocked-UV (Craver et al., 2014a; Williams et al., 2015), indicating that UV can regulate the expression of a series of genes that may prevent the development of intumescences. Based on this hypothesis, we overlapped the mis-expressed genes in the intumescent leaves to the genes regulated by UV. The results indicate there were 366 genes affected by both UV and intumescences, but only 11 genes were oppositely regulated (Fig. 7, Supplemental Table 4 and 5). Among them, 7 UV-induced genes were repressed, while 4 UV-repressed genes were induced in the intumescent leaves (Table 2). Interestingly, *3-beta-hydroxysteroid dehydrogenase (3β-HSD)*, an UV-inducible gene, was down-regulated in the intumescent leaves. Its isoform, *3-beta-hydroxysteroid dehydrogenase isoform 2 (3β-HSD2)* was also oppositely regulated between intumescent and healthy leaves during the development, suggesting that the *3β-HSD* family may play a key role in the intumescence development. These eleven genes may provide insight regarding how UV abates the development of intumescence; however, the function of most of these genes remains to be revealed.

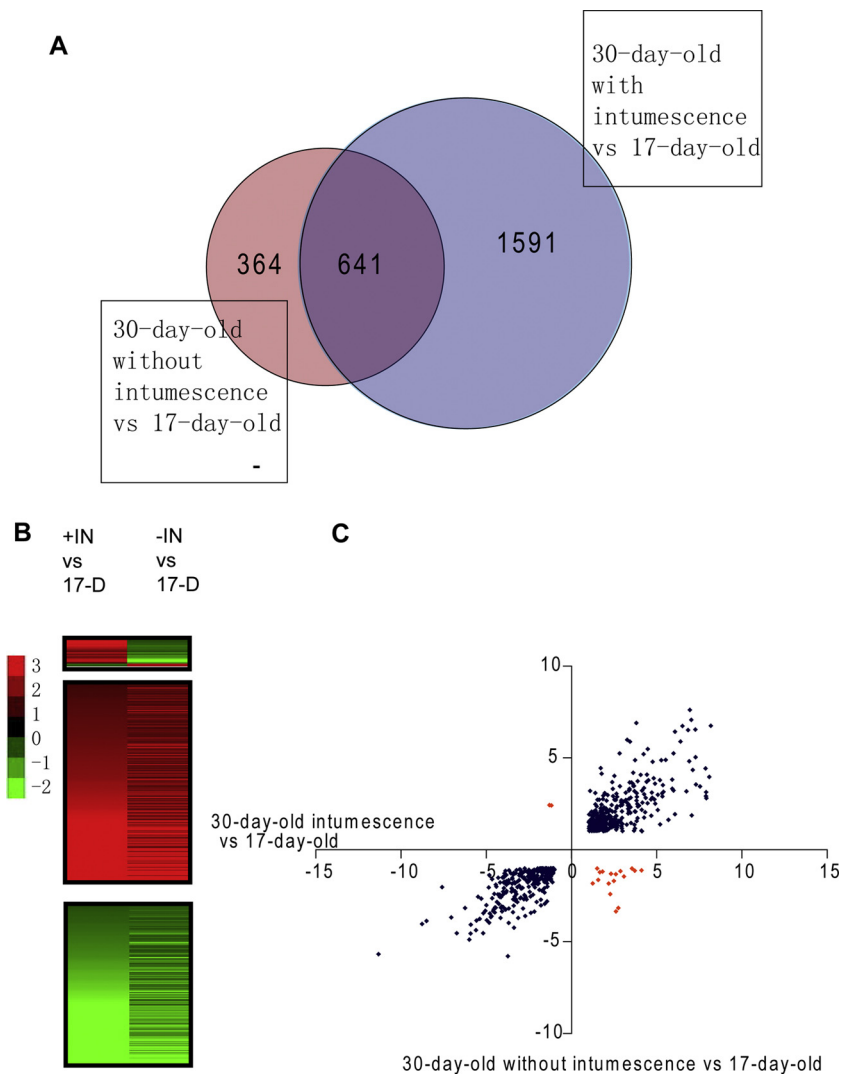


Fig. 6. The changed genes during the development of intumescent leaves and healthy leaves. (A) Venn diagram showing genes that were differentially expressed in the intumescent and healthy leaves during the development from 17-day-old to 30-day-old plants. (B) Cluster view of differentially expressed genes of 30-day-old intumescent or healthy leaves as compared with 17-day-old leaves, respectively. Most of the genes were positively correlated; however, 19 genes showed an opposite regulation pattern. Each row represents a single transcript. (C) Scatter plot showed the distribution of the changed genes of both intumescent and healthy leaves as compared with 17-day-old leaves, respectively, during the growing stage. The genes that showed positive correlation between intumescent and healthy leaves are shown in blue, and the genes showing opposite regulation patterns between intumescent and healthy leaves are shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.7. Validation of the microarray data by qPCR

Ten transcripts selected from Tables 1 and 2, including AY731066 (Major intrinsic protein subfamily), BI921925 (Xyloglucan endotransglycosylase hydrolase), and CK720557 (Acid phosphatase 1), were used for validation of the microarray data by qPCR. The results indicate that the data of qPCR are consistent with those of microarray (Fig. 8). The primers used in the qPCR research are listed in the Supplementary data Table S1.

4. Discussion

Previous research has suggested that intumescences can be induced by various environmental conditions; however, the fundamental physiological and molecular changes of plants

during the development of the disorder and the crosstalk between intumescences and UV radiation remain unknown. Here, we used microarray technology to investigate the changes of transcriptional profiles during the development of intumescences and treatment with UV radiation. The microarray data indicated that the hypertrophied cells encounter dramatic disorders in several important biological processes and signaling transduction. In addition, the data provided clues for the interactions between the intumescences and UV radiation at the molecular level.

Intumescences have been known to disorder multiple physiological aspects of plants, and one of the most detrimental impacts of intumescence is the impairment of photosynthesis. A physiological study has shown that impaired photosynthesis is correlated with the severity of abiotic leaf blotches (Roloff et al., 2004), and

Table 1

List of genes that show opposite regulation patterns between intumescent leaves and healthy leaves during the growing stage from 17- to 30-day-old. In most cases, annotation is based on the studied closest homolog/ortholog from other species. (For interpretation of relative gene expression in colour, the reader is referred to the web version of this article.)

Gene Number	Bank	Annotation	Relative expression compared with 17-day-old (log ₂ ratio)	
			Intumescent leaves	Healthy leaves
X58885.1		1-aminocyclopropane-1-carboxylate oxidase 4, Ethylene-forming enzyme	4.110694811	-1.124465582
CN385384		Gibberelin activated protein	3.54688298	-1.024429048
AW623381		Cell wall proteins	3.714163735	-1.142771273
AY731066.1		MIP (Major Intrinsic Protein) subfamily	2.87385827	-1.167332876
CK720557		Acid phosphatase 1	3.080298821	-1.456144828
BI921925		xyloglucan endotransglycosylase hydrolase	2.603786933	-1.333282034
CN385311		Dirigent-like protein	2.315658852	-1.31048322
BI204877		Translocon-associated protein	1.823106077	-1.192325801
BG631546		GDSL-motif lipase	1.505391793	-1.023575372
AF077339.1		Endo-beta-glucanase	2.486340974	-1.696857391
BG626664		Early-responsive to dehydration 3	1.663159141	-1.222248763
AW034232		Aspartyl protease family protein	2.179853371	-1.839656509
BI208286		2-oxoglutarate-dependent dioxygenase	1.566077523	-1.643981018
AI773603		Signaling protein	2.273314973	-2.415740281
AW737374		MYB domain transcription factor family	2.74902841	-3.174093172
Z46674.1		unknown	2.608750351	-3.358123339
AF184232.1		alpha-expansin precursor (Cell Wall)	1.241447461	-1.836883733
BT013803.1		3-beta-hydroxysteroid dehydrogenase isoform 2	-1.294093887	2.424786759
AW932956		3-hydroxy-3-methylglutaryl reductase	-1.177128287	2.408974674

Log₂ ratio

can be further explained by a reduction in the amount of tissue available for light absorption in intumescent leaves (Pinkard et al., 2006). Our results showed that the photosynthetic and photorespiration associated genes were predominantly repressed in the intumescent leaves. Interestingly, some previous studies also claim that the impairment of photosynthesis is possibly caused by reducing the number of chloroplasts within hypertrophied cells (La Rue, 1933; Eisa and Dobrenz, 1971; Lang et al., 1983). Thus, it is possible that the hypertrophied cells of intumescent leaves are programmed to express less photosynthetic genes and reduce chloroplast formation.

Along with other phytohormones, ethylene has been proposed to play a dominant role in the cellular hypertrophy and hyperplasia in plant tissues affected by intumescences (Petitte and Ormrod, 1986). Wallace (1928) first documented ethylene as a causative factor in lesion development on apple (Wallace, 1928). The study suggests that ethylene treatment resulted in a disorganization of

various tissues, indicating that cell wall digestion along with hypertrophy and hyperplasia of cells may potentially be induced by ethylene (Wallace, 1928). Similarly, ethylene-associated enations have also been observed in potato (Kirkham and Keeney, 1974). Our microarray results suggest that all the genes related with ethylene synthesis and signaling covered in the microarray were up-regulated in the intumescent leaves. Together with our results, over-synthesis and over-response of ethylene may contribute to the development of intumescences. However, further studies are needed to dissect either the synthesis or response of ethylene in intumescence development.

Our previous results show that intumescences can be induced by blocking UV (Williams et al., 2015), indicating that there is crosstalk between UV radiation and intumescences; however, the basic mechanism of this crosstalk still remains unknown. UV radiation functions as an environmental signal and can be perceived by plant systems (Robson et al., 2015).

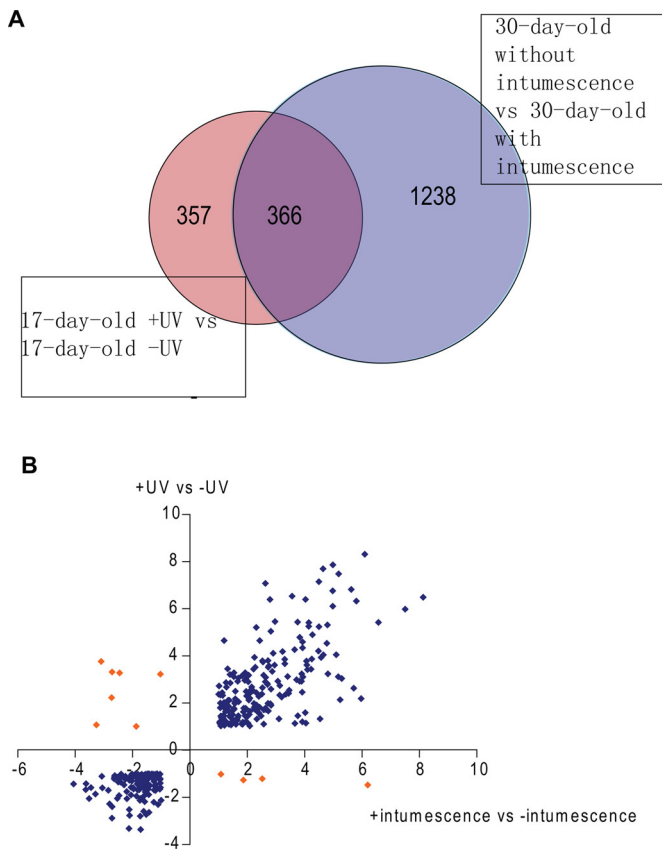


Fig. 7. The genes that were both affected by intumescence and UV radiation. (A) Venn diagram showing genes involved in both blocked-UV and UV treatment. (B) Scatter plots showing the changed genes involved in both intumescence and UV response. Among the overlapping genes, most genes show positive correlation; however, 11 genes show the opposite regulation pattern. The genes that showed a positive correlation pattern are highlighted in blue, and the genes showing an opposite regulation pattern are highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

List of genes that show opposite regulation patterns between genes regulated in intumescent leaves and regulated by UV treatment. In most cases, annotation is based on the studied closest homolog/ortholog from other species. (For interpretation of relative gene expression in colour, the reader is referred to the web version of this article.)

Gene Number	Bank	Annotation	Relative expression compared with 17-day-old (log ₂ ratio)	
			+UVB vs -UVB	With intumescence vs non-intumescence
BG631544		GH3.1	-1.478935368	6.198946889
BG625896		3-beta-hydroxysteroid dehydrogenase	1.069971573	-3.261502232
AW218727		unknown	-1.214002171	2.521713772
BG135595		unknown	1.008357802	-1.871288341
BT012714.1		pectate lyase	-1.265822303	1.864179804
AI483637		unknown	2.229507419	2.729183158
BG130169		extensin-like protein	-1.017310004	1.0792165
AW094303		unknown	3.763388415	-3.093880927
AJ320060		unknown	3.31768727	-2.713798295
AJ320060		unknown	3.275861085	-2.452033823
BT012962.1		bifunctional nuclease	3.227047744	-1.030152489



Plants have sophisticated mechanisms, such as turning on the flavonoid synthesis genes, to protect them from damage associated with UV exposure (Ulm et al., 2009). Interestingly, our microarray results show that the flavonoid synthesis genes were down-regulated in the intumescent leaves, suggesting that decreased accumulation of flavonoids may promote the development of intumescences. Therefore, induction of synthesis of the secondary metabolites, such as flavonoids, by UV might be one of the key factors that contribute to inhibiting the development of intumescences.

We have identified a series of potential genes that may play key roles in intumescence development (Table 1) and abatement of intumescence by UV radiation (Table 2). Most of the genes in the lists have not yet been functionally characterized. Interestingly, *3β-HSD* and *3β-HSD2* from one family were listed in both tables. The function of *3β-HSD* is to remove the two methyl groups at the C-4 position of cycloartenol, a precursor of plant sterols, which regulate membrane permeability and fluidity and further affect auxin transport (Kim et al., 2011, 2012). Over-expression of *3β-HSD* has been shown to cause abnormal auxin transport and resulted in growth defects (Kim et al., 2012). Auxin has been recognized as a potential causative factor for intumescence-like disorders (White, 1951). Thus, the mis-regulation of *3β-HSDs* in the intumescent leaves may cause abnormal auxin transport.

In conclusion, we identified a series of interesting genes, especially the photosynthesis-, flavonoid synthesis-, and ethylene-related genes, that were differentially regulated in the intumescent leaves, suggesting explanations for the phenotypic and physiological phenomena associated with intumescences. In addition, by seeking the genes that were oppositely regulated by UV radiation and intumescence, we identified some candidate genes that may help to explain how UV radiation can abate the occurrence of intumescence. This knowledge can facilitate mechanistic studies to further our understanding of the physiological disorder of intumescence.

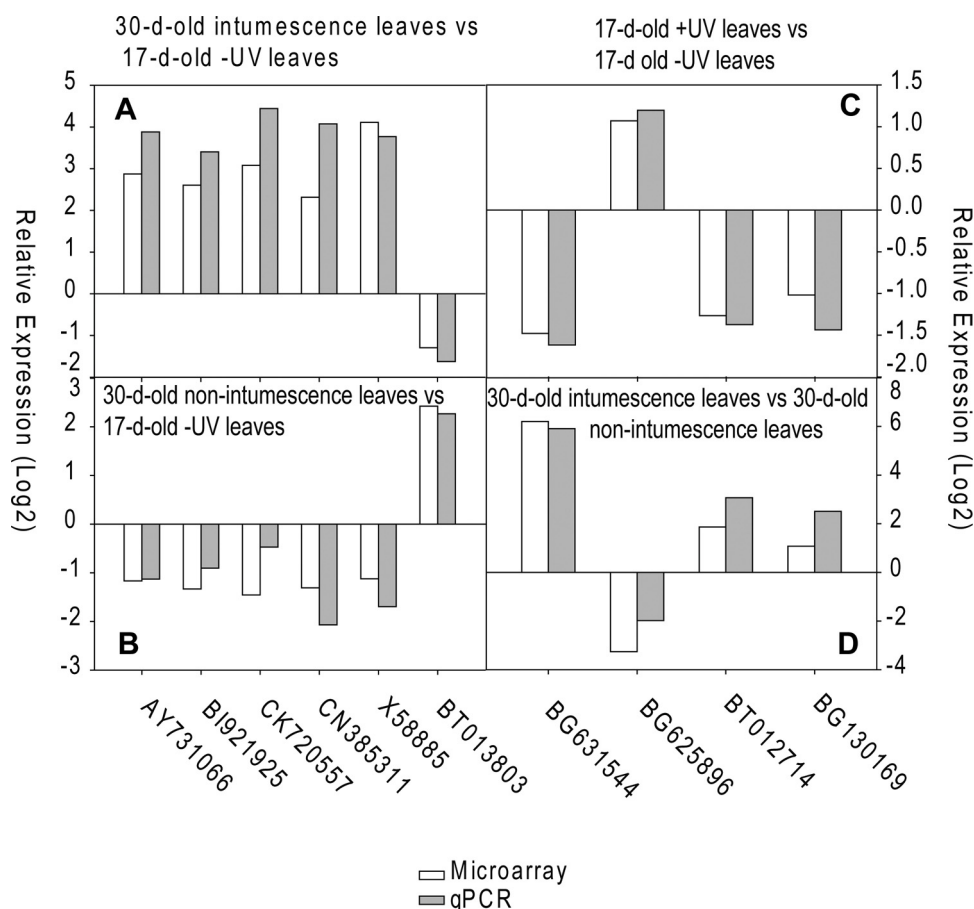


Fig. 8. Validation of the microarray results by qPCR. The x-axis represents the ID of the transcripts; the y-axis displays the relative gene expression assessed by qPCR and microarrays. (A) Comparison between the 30-d-old intumescent leaves and 17-d-old leaves grown under the blocked-UV condition; (B) comparison between the 30-d-old healthy leaves and 17-d-old leaves grown under the blocked-UV condition; (C) comparison between the 17-d-old leaves that were in the UV or blocked-UV treatments; (D) comparison between the 30-d-old intumescent and 30-d-old healthy leaves.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2016.11.006>.

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