Analysis of differential expression of Mediator subunit genes in Arabidopsis

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Mediator is a conserved eukaryotic multiprotein complex required by RNA polymerase II for transcription of its target genes. Till date, there is no report explaining the signals that affect the overall concentration of individual Med subunits. In this report, we have analyzed the effect of different phytohormones and stresses on the transcript level of *Med* genes in Arabidopsis. Hormones like auxin and JA, and cold stress did not show significant effect. ABA moderately increased the transcript abundance of more than 70% of *AtMed* genes analyzed in this study. However, there was noticeable change in the transcript level of several *AtMed* genes in response to BR. Stresses like high light, dark and salt also caused significant change in the transcript abundance of many *AtMed* genes. These data reveal that different environmental cues can affect stoichiometric concentration of Med subunits by affecting the transcription of their respective genes. This may, in turn, affect the overall arrangement of functional Mediator complex. This also suggests that some subunits may have some specific functions to play in response different signals.

Introduction

Mediator, a necessary component for RNA pol II mediated transcription of genes, was first discovered in yeast, and then subsequently was found in many fungi and metazoans.[1,2] In 2007, the first plant Mediator complex was biochemically purified from Arabidopsis cell suspension culture.[3] Following this, through bioinformatic analyses, subunits of Mediator complex were reported in many other plants including rice.[2,4] Thus, Mediator complex is found in all the eukaryotes ranging from unicellular yeast to multicellular humans and plants suggesting its emergence before divergence of plants, animals and fungi. Consistent to this, in yeast and metazoans, Mediator has been found to play crucial roles in basic process of transcriptional regulation of eukaryotic genes. The genetic, biochemical and structural studies revealed the modular nature of the Mediator, wherein all the subunits are arranged in four modules; Head, Middle, Tail and Kinase.[5,6] The ability of specific subunits of Mediator complex to interact with site-specific transcription factors and RNA Pol II make it suitable to play a role as a communication bridge between transcriptional regulators and transcriptional machinery. In yeast and animals, several nuclear receptors and nuclear receptor-like transcription factors have been found to target Med1, Med14 and Med15 for their function.[7-12] Transcription factor VP16 interacts with Med17 and Med25 for its transcriptional activity, whereas E1A and ELK1 require Med23.[13-16] Similarly, NANOG,

β-catenin and REST target MED12.[17-19] Subunits like Med5 and Med17 were shown to interact with RNA pol II and its associated GTFs.[20-23] By chromatin immunoprecipitation experiments, Mediator was shown to co-localize with RNA pol II at many promoters, and additionally also at activator-bound regulatory elements.[24-27] By virtue of its ability to interact directly with pol II, besides contributing a lot to the transcription initiation process, Mediator also plays important role during transcript elongation. A functional link between Mediator and elongation factor DSIF, physical interaction between Mediator and P-TEFb, and a functional commonality between MED31 and elongation factor SII suggest critical role of Mediator in the process of elongation of transcripts.[28-30] There are many reports suggesting functional interactions of Mediator with chromatin co-factors. Acevedo et al. (2003) demonstrated synergistic function of Mediator and p300 during ERα-dependent transcription.[31] Mediator can interact with both p300 and TFIID, and so might be coordinating the exchange of chromatin modifying co-factors and PIC components.[32] This view is further strengthened by the observation of close association between Mediator and SAGA complex.[33,34] There is a possibility that Mediator is also controlling epigenetic pathways as it was found in a complex of REST-Mediator-G9a.[35] REST is a silencing factor and G9a is a methyltransferase, which methylates H3K9 at target genes.[19]

In plants, functions of some of the Mediator subunits have been established. In Arabidopsis, Med14, originally identified

as SWP, affects cell number and shoot meristem development.[36] AtMed14 coordinates with HDAC19 for its function.[37] Moreover, AtMed14 might have a role in splicing as overexpression of its cDNA could complement the *smp* mutant phenotype.[38] Other Mediator subunits like AtMed12, AtMed13 and AtCdk8 are also required for regulation of cell differentiation and development of plant.[39-41] The multifaceted AtMed25/PFT1 is involved in regulation of flowering time in response to light quality, hormonal signaling, and biotic or abiotic responses, by the virtue of its ability to interact with several key transcriptional regulators.[42-44] Three Arabidopsis Mediator subunits AtMed17, AtMed18 and AtMed20 were found to regulate levels of miRNAs.[45] Authors suggested that in plants, Mediator might also be cooperating with other RNA polymerases. Consistent with this, AtMed36 subunit was co-purified with the largest subunit of RNA Pol V.[46] Moreover, AtMed36 was predicted to be Fibrillarin which is involved in processing of rRNA.[47] The molecular mechanism by which specific plant Mediator subunits regulate specific processes is not known. However, as the case of AtMed25 suggests, different subunits might be targeted by different activators and repressors for their respective transcriptional responses. Moreover, interaction of different specific subunits with the components of transcriptional machinery might be conveying the transcriptional signals to the RNA polymerase.

Thus, in all the eukaryotes, the main function of Mediator complex is to regulate the process of transcription. In such scenario it is logical to suggest that abundance of Med subunits can affect the overall composition of the complex, and hence the transcriptional response. We do not know if developmental or environmental signals affect the level of Med subunits and the resultant complex. There is no comprehensive report on transcriptional regulation of *Med* genes coding for Mediator subunits in any organism. In this report, we have tried to analyze the regulatory promoter elements of the *Med* genes, and studied the effect of different environmental cues on the transcript level of *Med* genes in Arabidopsis.

Results and Discussion

Gene structure and chromosomal distribution of Mediator subunit genes in Arabidopsis. Structure of all the genes coding for Mediator subunits in Arabidopsis were obtained from TAIR. The Mediator subunit genes contain variable number of introns ranging from 0 to 27. About 20% genes in Arabidopsis are intronless, but we found only one such Med subunit encoding gene, *AtMed4*.[48] The *AtMed2* and *AtCdk8* genes have simple structure with one large intron, whereas *AtMed35* has 27 introns. All the other Med subunit encoding genes in Arabidopsis were found to contain ≥ 2 introns (Fig. S1) suggesting their gradual evolution unlike rapid evolution of intronless genes by duplication or reverse transcription/integration.[48] The intron/exon arrangement of all the *Med* genes in Arabidopsis are shown in Figure S1.

Location of Med subunit encoding genes mapped on Arabidopsis chromosome pseudomolecules available at TAIR is shown in **Figure 1A**. The exact positions and orientations of all the *Med* genes in Arabidopsis chromosome pseudomolecules

are mentioned in Table S1. The *AtMed* genes were found to be scattered randomly on the chromosomes of Arabidopsis. Chromosome 1, which is the biggest one, code for 17 Med subunits, whereas the smallest chromosome (chromosome 5) harbour five *Med* genes. Thus, more than 36% of *Med* genes are located on chromosome 1. The interesting feature on chromosome 1 is presence of four *Med15* paralogs (out of 5) in a span of just 13138 bases in the order of *Med15b-Med15c-Med15a-Med15d* (**Fig. 1B**). In this *Med15* cluster, intergenic distance between *Med15d* and *Med15a* is 1887 bases, *Med15a* and *Med15c* is 622 bases, whereas *Med15c* is separated from *Med15b* by an intergenic region of just 240 bases. In Arabidopsis, among all the Med subunits, we found that Med15 has the maximum number of paralogs (**Fig. 1A**, Table S1). In fungi and metazoans, Med15 is required by several transcription factors for their transcriptional responses.[9,11,12,49] Thus, in Arabidopsis, more number of Med15 paralogs might help in broadening the regulatory capability of Mediator complex by interacting with diverse transcription factors. In order to see if all these *Med15* genes are expressed or not, we performed RT-PCR analysis and found that all the paralogs being expressed in one or the other tissues of Arabidopsis (Kumar and Thakur, in preparation). It will be interesting to study the functional relevance of these expressed *Med15* genes.

***In silico* analysis of *Med* gene promoters.** Since promoters dictate the expression pattern of a gene, we analyzed promoter regions of *Med* genes in Arabidopsis. The putative 2 kb *AtMed* promoter sequences were identified *in silico*. Bioinformatic analyses of these sequences revealed presence of highly represented regulatory elements known in plant gene promoters, including elements responsive to hormones and stresses. Most of these elements were present within 1 kb promoter. **Figure 2** shows number of Arabidopsis *Med* genes carrying a particular responsive element in their promoter regions. Elements like ACGT, REalpha and I-box, responsible for response to light or darkness, were found to be present in more than 20 promoters of *AtMed* genes. Although in less number of *AtMed* genes, elements important for response to hormones like auxin (ARF, ASF1, AuxRE and SAUR), ABA (ABRE) or JA (T/G box and GCC core), and stresses like cold (LTRE) or salinity (SCAM) were also found. In order to understand the significance of presence of these elements in the upstream regulatory regions of *AtMed* genes, we studied the effect of hormones and stresses on the transcript abundance of 31 *Med* genes in Arabidopsis.

Hormonal regulation of transcription of *AtMed* genes. phytohormones play very important role in almost all the aspects of plant growth and development. They regulate transcription of a plethora of plant genes. In order to know if auxin affected abundance of Med subunits, we treated Arabidopsis seedlings with IAA and analyzed the change in transcript level of *Med* genes. Change in transcript level of *Aux2*-27 was used as positive control for auxin treatment.[50] As evident from **Figure 3A**, very few *AtMed* genes were affected by IAA. Moreover, the effect on these few genes was not so robust. Three *AtMed* genes, *AtMed12*, *AtMed4* and *AtMed31*, showed slight increase (> 1.5 fold) in their transcript levels, whereas *AtMed15*, *AtMed5*, *AtMed13* and *AtMed14* showed > 40% reduction. Interestingly,
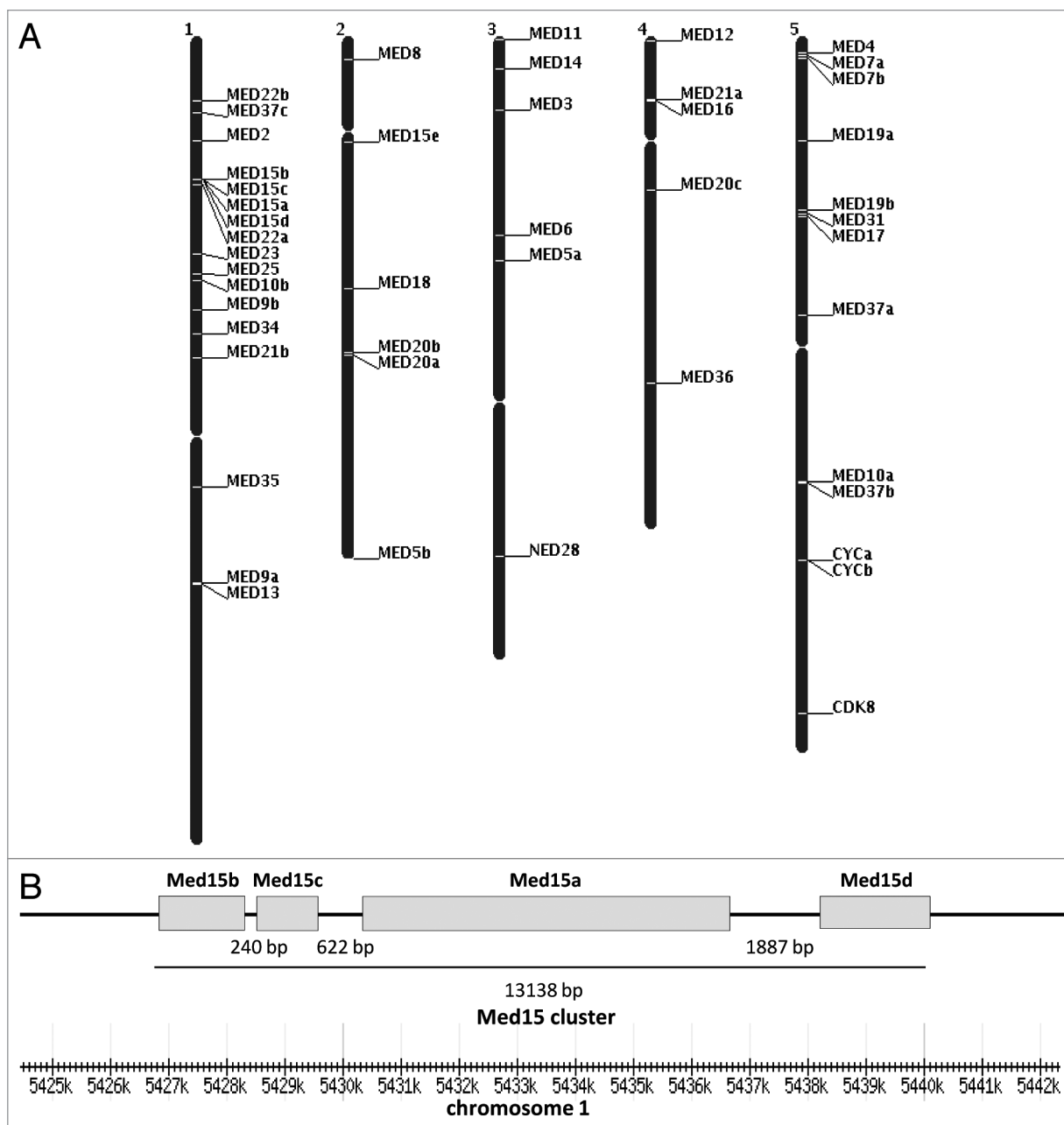
Figure 1. (A) Genomic distribution of *Med* genes on Arabidopsis chromosomes. Chromosome numbers are indicated at the top of the black bars which represent chromosomes. (B) Arrangement of *AtMed15* genes in the Med15 cluster on chromosome 1. Total length of Med15 cluster and intergenic distances between different *AtMed15* genes are shown in bp. Scale at the bottom shows distance from the telomeric end.

AtMed15 was severely reduced in the presence of auxin (**Fig. 3A**). It will be interesting to see how reduction in the level of this paralog of AtMed15 helps in auxin signaling. Just like auxin, brassinosteroid (BR) is another phytohormone that play very critical role in growth and differentiation of plants. A defect in BR signaling pathways can make plants dwarf and sterile. In order to understand the effect of BR on genes coding for AtMed subunits, change in their transcript levels in the Arabidopsis seedlings grown in the medium with BR were compared with that in the normally grown seedlings. Increase in transcript level of *SAUR9* by BR was used as control.[51] Surprisingly, unlike

that in the case of auxin, several *AtMed* genes were significantly upregulated in the presence of BR (**Fig. 3B**). Transcription of six *AtMed* genes, *AtMed6*, *AtMed17*, *AtMed9*, *AtMed15*, *AtMed12*, and *AtMed37*, were increased by more than 2.5 fold. Some other *AtMed* genes were moderately upregulated (eight; more than 1.5 fold), although four (*AtMed20*, *AtMed22*, *AtMed7* and *AtMed16*) were found to be slightly downregulated (less than 0.6 fold). Thus, although BR and auxin are known to act synergistically, expression of *AtMed* genes are affected differently by these two important plant hormones. This observation is significant because almost 40% auxin regulated genes are also affected by
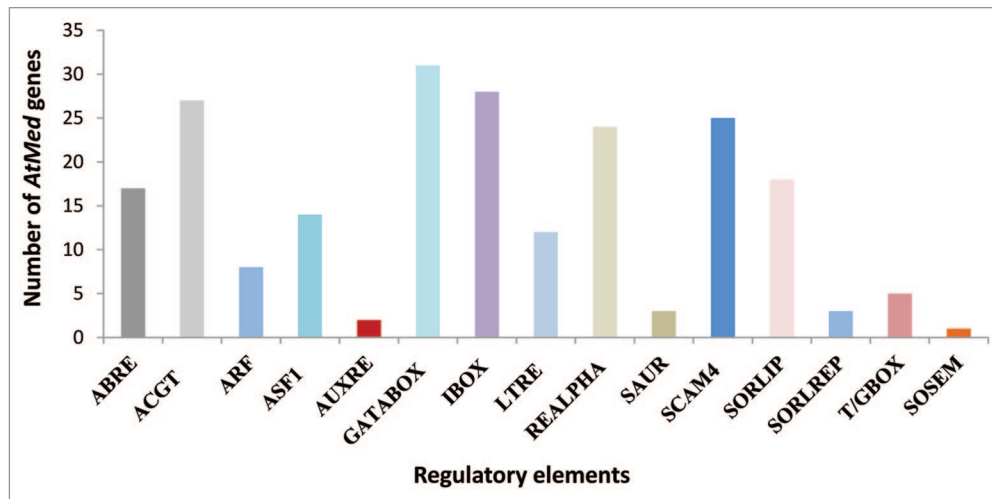
Figure 2. Total number of *AtMed* genes (y-axis) harboring a particular responsive element (shown on x-axis) in its promoter region. ABRE, ACGT, GATABOX (GATA box), IBOX (I box), REALPHA (REalpha), SORLIP and SORLREP are light/dark responsive elements. ARF, ASF1, AUXRE and SAUR are important for auxin response. ACGT and SOSEM represent ABA responsive elements. LTRE is responsive to cold and ABA. SCAM4 is important for response to salinity stress.

BRs, and many BR-regulated promoters have been found to harbour auxin responsive elements.[52,53] There is a possibility that these hormones give signals for different arrangement of Med subunits for their transcriptional outputs.

Phytohormone, abscisic acid (ABA), in addition to many growth and development related processes, is important for plants in response to several abiotic stresses. When Arabidopsis seedlings were grown in media with ABA, not much of the significant effect was observed in transcript level of *Med* genes, though transcript levels of many were increased by more than just 1.5 fold (**Fig. 4A**). These moderately upregulated *Med* genes were *AtMed17, 9, 37, 25, 7, 20, 23, 15, 3, 18, 14, 31, 4, 22,* and *AtCdk8*. In the case of ABA, not a single *AtMed* gene was downregulated. Relative expression of *AtHsf* was used as a positive control for effect of ABA on gene expression. Jasmonic acid (JA) is a jasmonate class of plant hormone that promotes plant defense responses against pathogen attack and physical wound. In order to know if JA changes the level of any Med subunit, we analyzed the transcript levels of 31 *Med* genes in the Arabidopsis seedlings grown in the agar media with JA (**Fig. 4B**). Upregulation in the transcript level of *JAZ* in comparison to the seedlings grown in normal agar medium without JA was used as control.[54] *AtMed15* and *AtMed36* showed little more than 1.5 fold increase in the transcript level, whereas *AtMed18* showed more than 2 fold increase (**Fig. 4B**). On the other hand, transcript levels of *AtMed12*, *AtMed21* and *AtMed37* decreased in the seedlings grown in JA. All other *AtMed* genes did not show any difference in their transcript abundance in response to JA treatment. Thus, except *AtMed18* as in the case of JA, no other *AtMed* gene showed significant upregulation in response to two main stress response regulating hormones, JA and ABA. In Arabidopsis, partial loss of function of *AtMed18* causes developmental defects like shorter petioles, abnormal phyllotaxy, late flowering, abnormal flowers, and reduced fertility.[45] These phenotypes are regulated by signaling pathways evoked by diverse plant hormones. It will

be interesting to see if hormones, like JA, require AtMed18 for these functions.

Effect of light on the transcription of *AtMed* genes. Being sessile organisms, plants possess a well organized system to assess the quality of light and utilize it for their growth and survival, and to modulate different developmental programmes including flowering. In order to see if light affected stoichiometric concentration of Med subunits in plants, we analyzed their transcript abundance in Arabidopsis seedlings either treated with more than normal range of light intensity or grown in complete darkness. In etiolated seedlings, transcription of *AtMed15* and *AtMed37* was significantly upregulated, more than 2-fold, in comparison to normally grown seedlings under 14 h light and 10 h dark cycle (**Fig. 5A**). Additionally, there was slight increase (> 1.5 fold) in the transcript levels of *AtMed14* and *AtMed19* in these seedlings. Interestingly, more number of *AtMed* genes were downregulated in dark. Transcript levels of *AtMed22*, *AtCycC*, *AtMed36*, *AtMed17*, *AtMed3*, *AtMed20*, *AtMed25*, *AtMed11*, *AtMed21* and *AtMed18* were reduced to half or less in etiolated seedlings in comparison to that in normally grown seedlings (**Fig. 5A**). Arabidopsis belongs to the category of long day plants, so it is sensitive to complete darkness. This might be the reason that it downregulates its transcriptional machinery when grown in complete darkness. Consistent with the observation in etiolated seedlings, many *AtMed* genes were upregulated when normally grown Arabidopsis seedlings were subjected to high light intensity (150 W m⁻²) for 3 h. Transcript level of five genes, *AtMed12*, *AtMed25*, *AtMed17*, *AtMed23* and *AtMed15*, increased more than 2-fold in response to high light, though nine more *AtMed* genes were moderately upregulated, more than 1.5 fold and less than 2 fold (**Fig. 5B**). Thus, presence and absence of light, which is an essential requirement for plant growth and development, affect expression of most of the *AtMed* genes. It is noticeable that, except *AtMed15* and *AtMed37*, all other *Med* genes were either upregulated by high light or downregulated by dark. Surprisingly,
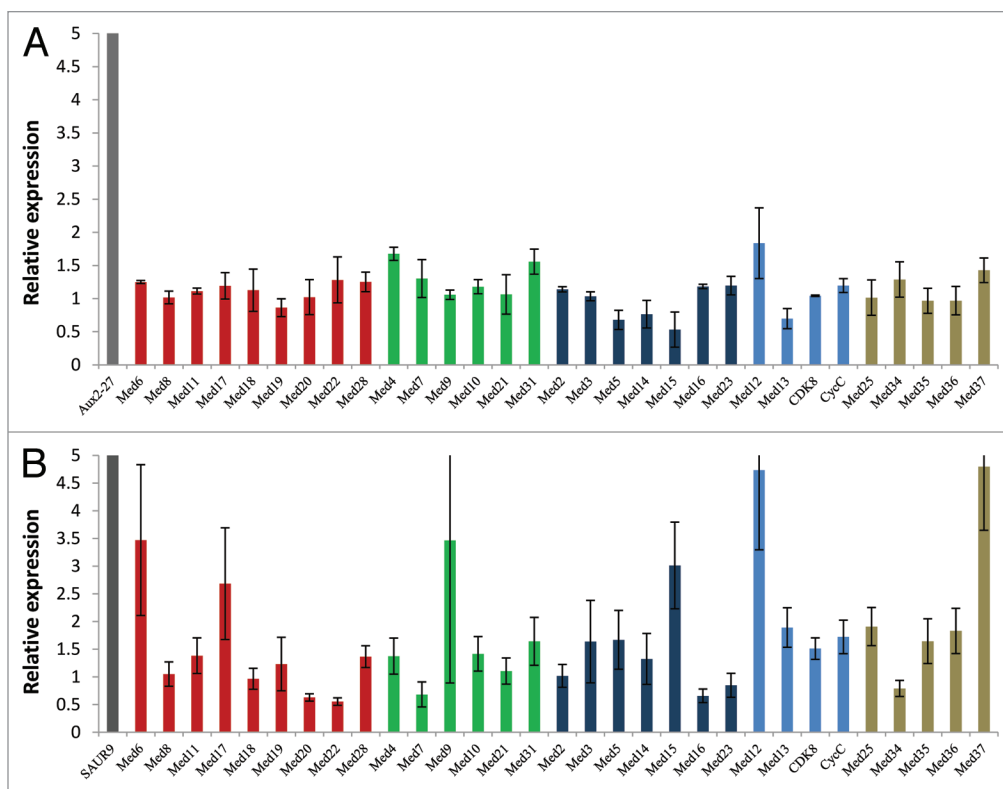
Figure 3. Quantitative real-time RT-PCR analysis showing change in the transcript abundance of *Med* genes in Arabidopsis seedlings treated with (A) auxin or (B) brassinosteroid. *Aux2–27* and *SAUR9* were used as positive controls for respective treatments. The relative expression level represents fold change to control seedlings. Mean values from three independent experiments are shown. Error bars represent standard deviation. Red, green, dark blue and blue bars represent *AtMed* genes coding for subunits of Head, Middle, Tail and Kinase modules, respectively. Subunits, whose positions are not known, are shown in tan color.

AtMed15 and *AtMed37* were found to be positively affected by both the high light and complete darkness. There is a possibility that transcription factors required for transcriptional response of light/dark might be targeting them for their response. In fungi and animals, Med15 subunit has been found to interact with several transcription factors. According to bioinformatic analyses, Med37 seems to be a plant specific subunit, suggesting its importance in light/dark mediated plant specific process. As mentioned earlier, we used high intensity of white light for this study. Since different photoreceptors perceive different wavelengths of light to regulate complex photomorphogenic events in plants, it will be interesting to study the role of Med subunits in UV, red, far-red and blue light responses.

Stress mediated changes in the transcription of *AtMed* genes. Plants face so many stresses in their lifetime. In order to cope up with these stresses, they have different network of gene expression. We analyzed effect of cold or salt on the transcription of *AtMed* genes. In the Arabidopsis seedlings kept in cold for 24 h, transcript level of *AtMed37* increased more than 2.5 folds whereas that of *AtMed18* and *AtCycC* decreased 2-fold (**Fig. 6A**). Three *AtMed* genes, *AtMed20*, *AtMed22* and *AtMed23*, were slightly downregulated. There was not much effect on any other *AtMed* gene. Even, transcription of *AtMed16*, which was characterized as *SENSITIVE TO FREEZING6* (*SFR6*) having prominent role in cold acclimation, was not affected by cold.[55] Increase in *Cor15A*

transcript was used as a positive control to study the effect of cold.[56] A number of literatures exist which explains transcriptome profile of different plants including Arabidopsis, in response to NaCl treatment which is used as a laboratory method to mimic stress caused by salinity in the soil or water used for irrigation.[57] In our analysis, five *AtMed* genes (*AtMed37*, *AtMed16*, *AtMed20*, *AtMed12* and *AtMed4*) showed more than 2-fold increase in their transcript abundance in response to presence of NaCl in the media, whereas 13 other *AtMed* genes were moderately upregulated by 1.5 fold or more (**Fig. 6B**). Expression of *Cor15A* was assessed as the control for salinity stress.[58] A couple of *AtMed* genes, *AtMed3* and *AtMed34*, showed slight reduction in their transcript level under salt stress. Thus, just like ABA response, a big number of *AtMed* genes were found to be upregulated by NaCl in Arabidopsis. This supports the fact that ABA plays important role in response to salt stress. Significant overlap in the *Med* genes showing increased transcription in the presence of ABA or salt indicate a possibility, that during salt or other stress, ABA maintains the increased concentration of functional Mediator complex by increasing the abundance of most of the subunits.

Conclusion

In plants, since the initial purification and characterization of Mediator in 2007, a number of reports have emerged revealing the
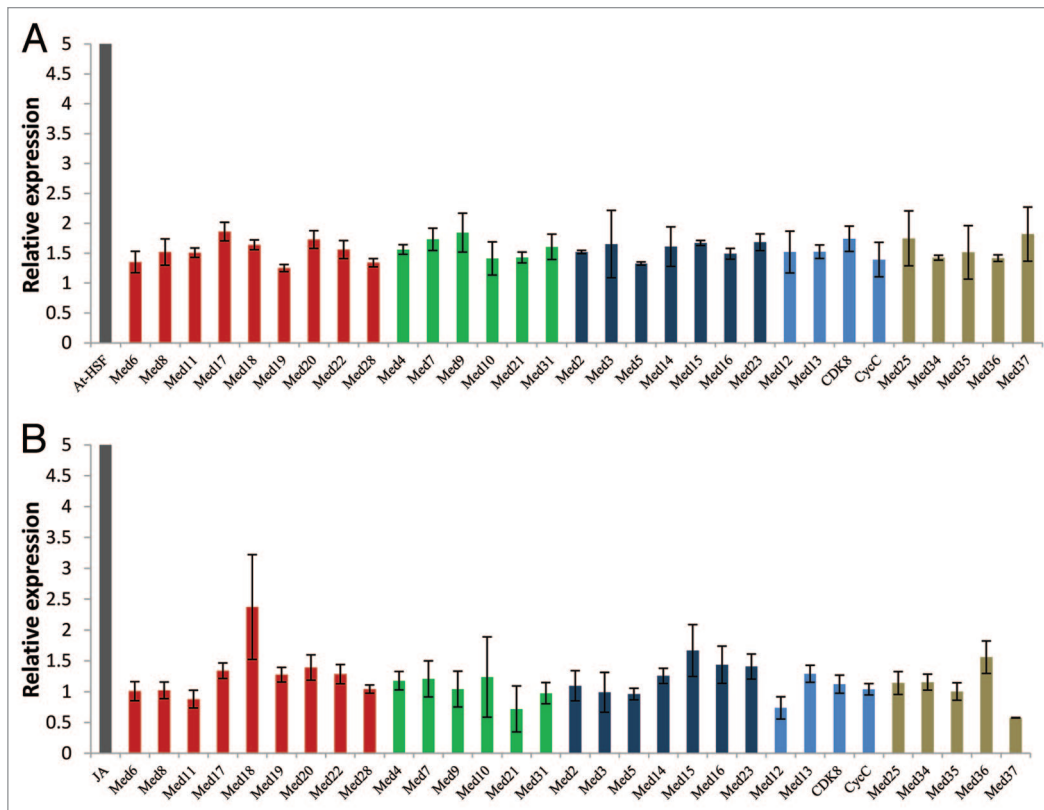
Figure 4. Quantitative real-time RT-PCR analysis showing change in the transcript abundance of *Med* genes in Arabidopsis seedlings treated with (A) abcisic acid or (B) jasmonic acid. *AtHSF* and *JA* were used as positive controls for respective treatments. The relative expression level represents fold change to control seedlings. Mean values from three independent experiments are shown. Error bars represent standard deviation. Red, green, dark blue and blue bars represent *AtMed* genes coding for subunits of Head, Middle, Tail and Kinase modules, respectively. Subunits, whose positions are not known, are shown in tan color.

role of the subunits of this complex in diverse processes like hormonal signaling, plant growth and development, flowering, and response to different biotic and abiotic stresses.[42] Thus, just like in fungi and metazoans, in plants too, several pathways converge on Mediator. There is no information on the direct effect of these pathways on the transcriptional regulation of *Med* gene expression. As summarized in **Figure 7**, in this study, some hormones like BR and ABA, and stresses like high light, darkness and salinity, affected transcript abundance of many *AtMed* genes. On the other hand, hormones like auxin and JA, and cold stress did not affect so many *AtMed* genes. To our knowledge, this is the first such study on *Med* genes in any eukaryotic organism. We found that BR signaling had significant impact (increase) on the transcript level of at least six *AtMed* genes; two coding for subunits of Head module, one for each of the other modules. Product of *AtMed37*, which is highly upregulated by BR, has not yet been assigned to any module. It will be interesting to find out if these subunits can make a functional core structure or they provide the peripheral variation to the interface, to play some role in transcriptional response of BRs. We can however, not rule out the specific role of these subunits in BR signaling. A similar observation was made in the case of salinity stress. In another scenario, ABA moderately upregulated more than 70% of tested *Med* genes in young Arabidopsis seedlings (**Fig. 4A and Fig.** 7). Many reports have suggested that

among all the hormones, ABA affects maximum number of plant genes (> 10% of protein coding genes in Arabidopsis) at the transcript level suggesting its pivotal role in plants.[59,60] Thus, there is requirement of more transcriptional machineries to meet the demand. Our results suggest that ABA increases the overall concentration of Med subunits to form more Mediator complexes to cover all the target promoters. Consistent with this, we made similar observation in the case of Arabidopsis seedlings growing in the presence of NaCl mimicking salinity stress that use ABA pathway for its transcriptional responses. One other striking observation was downregulation of almost 68% of tested *Med* genes in etiolated Arabidopsis seedlings (**Fig.** 7). Seedlings grown in complete darkness adopt a skotomorphogenic program of development by actively repressing transcription of most of the genes to conserve the reserved energy. Thus, it seems that since transcriptional activities are down, expression level of *Med* genes coding for Mediator complex, an important component of transcriptional machinery, are also down in the dark grown seedlings. On the other hand, exposure of etiolated seedlings to light is known to induce massive reprogramming of the transcriptome.[61] Transcript abundance of more than 20% of Arabidopsis genes increased when etiolated seedlings were irradiated with light for just about one hour.[62,63] Thus, it is not surprising that more than 45% of *AtMed* genes are upregulated in response to high light (**Fig.** 7). Arabidopsis and
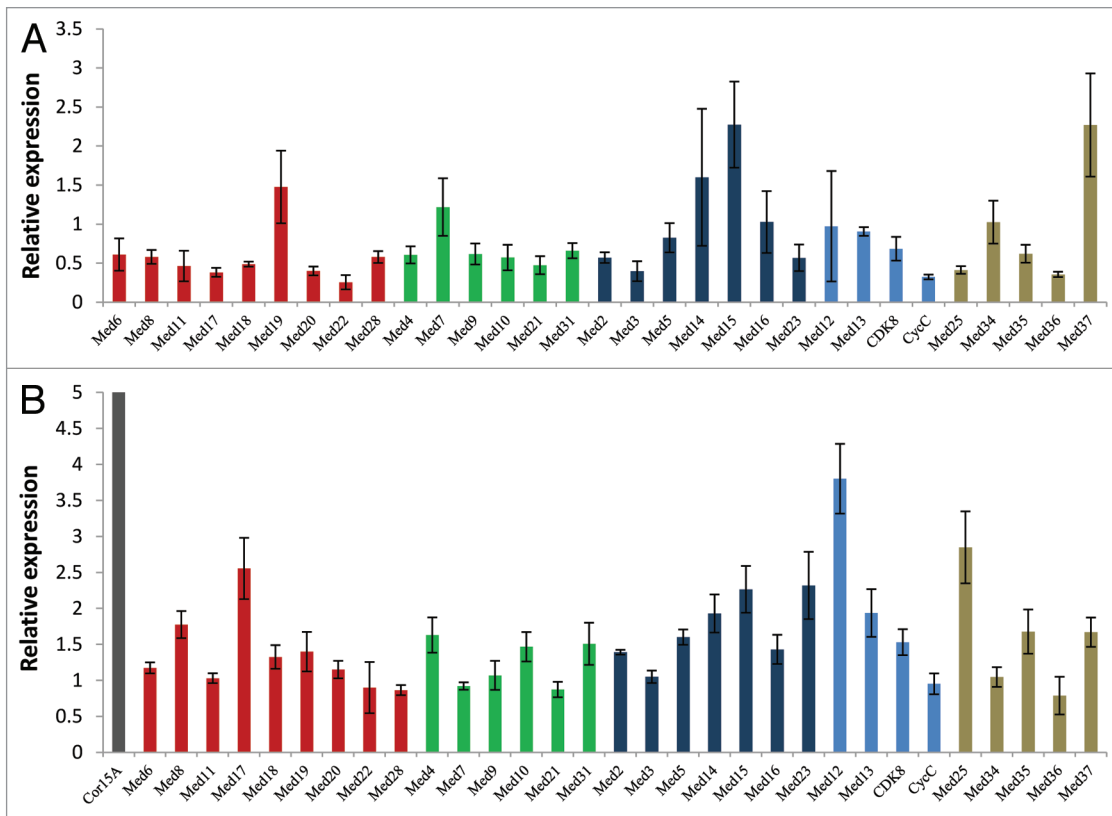
Figure 5. Quantitative real-time RT-PCR analysis showing change in the transcript abundance of *Med* genes in Arabidopsis seedlings (A) grown in complete darkness or (B) treated with high light. *Cor15A* were used as positive control for high light stress treatment. The relative expression level represents fold change to control seedlings. Mean values from three independent experiments are shown. Error bars represent standard deviation. Red, green, dark blue and blue bars represent *AtMed* genes coding for subunits of Head, Middle, Tail and Kinase modules, respectively. Subunits, whose positions are not known, are shown in tan color.

other land plant cells require light for growth and differentiation. We hypothesize that in complete darkness, Arabidopsis downregulates its Mediator and transcriptional machinery. When etiolated seedlings are exposed to light, components of transcriptional machinery including the Med subunits, are increased to fulfil the demand of increased transcriptional activity to support the photomorphogenic development. Earlier, in mammalian study, a drastic reduction and nearly complete loss of Mediator subunits was reported in differentiated myotube cells.[64] This suggests that the function of Mediator in differentiation is conserved in plants and animals. As analyzed in the Venn diagramme, many *AtMed* genes were upregulated by more than one treatment (**Fig. 8A**). This suggests that different signaling pathways merge on one or more Med subunits. Contrarily, very less number of AtMed genes was downregulated by two or more individual treatments (**Fig. 8B**). Thus, different signaling pathways seem to merge on Med subunits preferably by increasing its stoichiometric level in the complex, confirming the role of Med complex as an integrative hub for diverse signaling cascades.

Considering intrinsic modularity of the complex, variation may arise in the function of the plant Mediator complex. One level of heterogeneity may arise due to preferential incorporation of one of the subunit paralogs, into the functional complex. For example, replacement of one AtMed15 paralog with any other

four remaining paralogs. Additional variation may arise from overexpression or substoichiometric expression of a given subunit or a set of subunits relative to other Med subunits. The factors, including hormones or stresses, affecting the expression level of Med subunits, may thus, be affecting the overall composition of functional Mediator complex, making composition and arrangement of the subunits a context specific response.

Materials and Methods

Plant materials and treatments. *Arabidopsis thaliana* wild-type seeds (ecotype Columbia Col 0) were sterilized and then sown on MS-agar (Murashige and Skoog medium supplemented with 0.8% agar and 2% sucrose) plates. Seeds were stratified in dark for 48 h at 4°C, and then transferred to growth room. The seedlings were grown at 22°C for 10 d under 16 h light and 8 h dark cycle. According to the experimental requirements, 10 d old seedlings were transferred to MS-agar with 25 μM ABA or 30 μM JA or 200 mM NaCL, and grown for another 3 d. For cold stress treatment, 10 d old seedlings on MS-agar plates were incubated at 4°C for 24 h. IAA and BR treatments were given in liquid medium. For this, 10 d old seedlings were transferred to a flask containing liquid MS medium supplemented with 10 μM IAA or 1 μM Epibrassinolide, and then were shaken at 100 rpm for
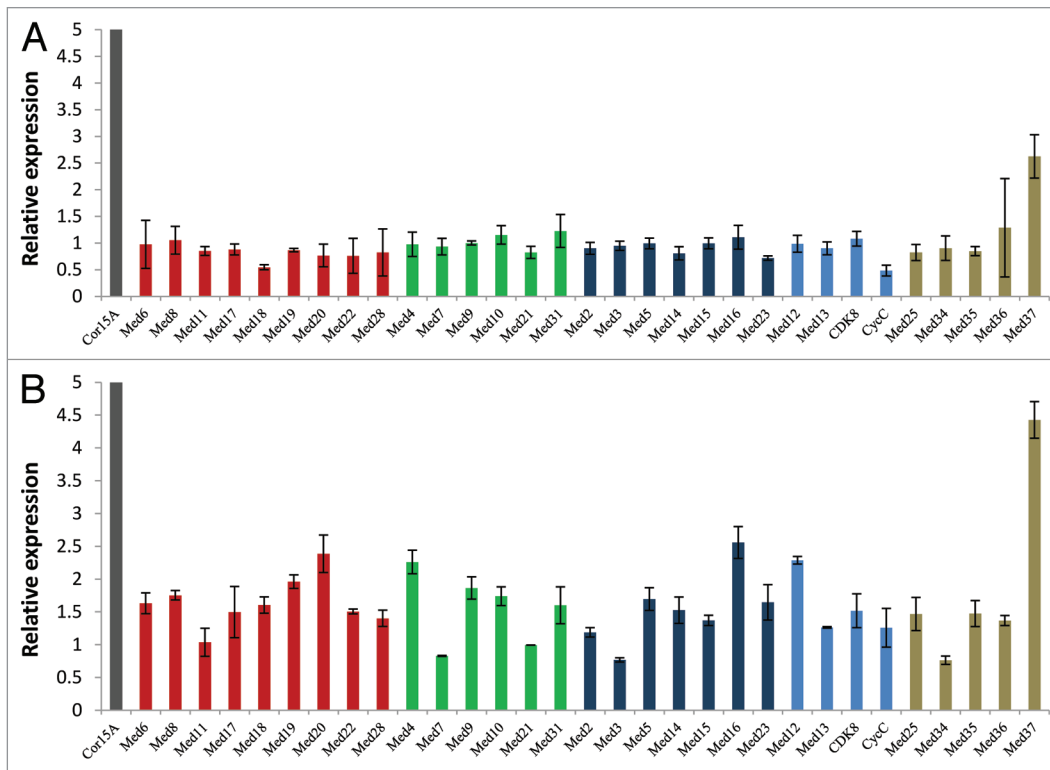
Figure 6. Quantitative real-time RT-PCR analysis showing change in the transcript abundance of *Med* genes in Arabidopsis seedlings subjected to (A) cold stress or (B) salinity stress treatment. *Cor15A* was used as positive control for both the stresses. The relative expression level represents fold change to control seedlings. Mean values from three independent experiments are shown. Error bars represent standard deviation. Red, green, dark blue and blue bars represent *AtMed* genes coding for subunits of Head, Middle, Tail and Kinase modules, respectively. Subunits, whose positions are not known, are shown in tan color.

3 h. For light stress treatment, 10 d old seedlings were incubated under high light ($800\mu E\ m^{-2}\ s^{-2}$) in phytotrone (PGV36) for 3 h. After required growth and treatments, seedlings were harvested, immediately frozen in liquid nitrogen and then stored in -80°C till further use.

Isolation of RNA and reverse transcription. Total RNA was isolated from Arabidopsis seedlings using Spectrum Plant Total RNA Kit (SIGMA Life Sciences) following the manufacturer's instructions. The amount and quality of RNA was checked by Nano Nanodrop ND-1000 Spectrophotometer (Thermo SCIENTIFIC) and further confirmed by electrophoresis in 1.2% formamide agarose gel. For each sample, 5μg of total RNA was reverse transcribed to cDNA in 33 μl reaction using First- Strand cDNA Synthesis Kit (GE Healthcare). Reverse transcription were performed at 37°C for 1 h. 50 times diluted cDNA mix was used for qPCR. qPCR reaction included SYBR Green Master Mix (Applied Biosystem), a gene specific primers and 2μl of the 50 times diluted cDNA. List of primers used in this study are given in Table S2.

Real time PCR analysis. β-Actin was used as an internal control. Primers for all the genes were designed by using coding sequences of Arabidopsis genes available on TAIR database http:// www.arabidopsis.org/ by using the online available software Integrated DNA Technologies Oligo Analyzer3.1 for calculating the Tm and GC content of primers. Details of primers used in this

study are given in Table S2. The reaction conditions were: (95°C for 5 min, followed by 40 cycles at 95°C for 30 sec and 60°C for 60 sec, and then a final extension of 72°C for 5 min). qPCR was performed and analyzed in 7900 HT Real-Time PCR Detection System (Applied Biosystem). At least three biological replicates have been analyzed for every gene. Three technical replicates have been employed for each biological replicate. The threshold cycles (Ct) of each tested genes were averaged for triplicate reactions and the values were normalized to endogenous control of β-Actin gene (Ct Target gene – Ct Endogenous control) then normalized to reference sample for calculating the fold change.

Prediction of *AtMed* gene promoters and mapping of genes on Arabidopsis chromosomes. The promoter sequences (2000bp upstream 5'UTR region) were obtained from the sequence of BAC clones carrying the *AtMed* genes. The BAC sequences were obtained after BLASTX search on NCBI database (http://blast.ncbi.nlm.nih.gov) using cds of *AtMed* genes as the queries. The PLACE Database available on www.dna. affrc.go.jp was used to get different abiotic stress- and hormone-responsive elements. The TAIR based chromosome map tool www.arabidopsis.org/ was used to find the location of all the Mediator genes.

Disclosure of Potential Conflicts of Interest

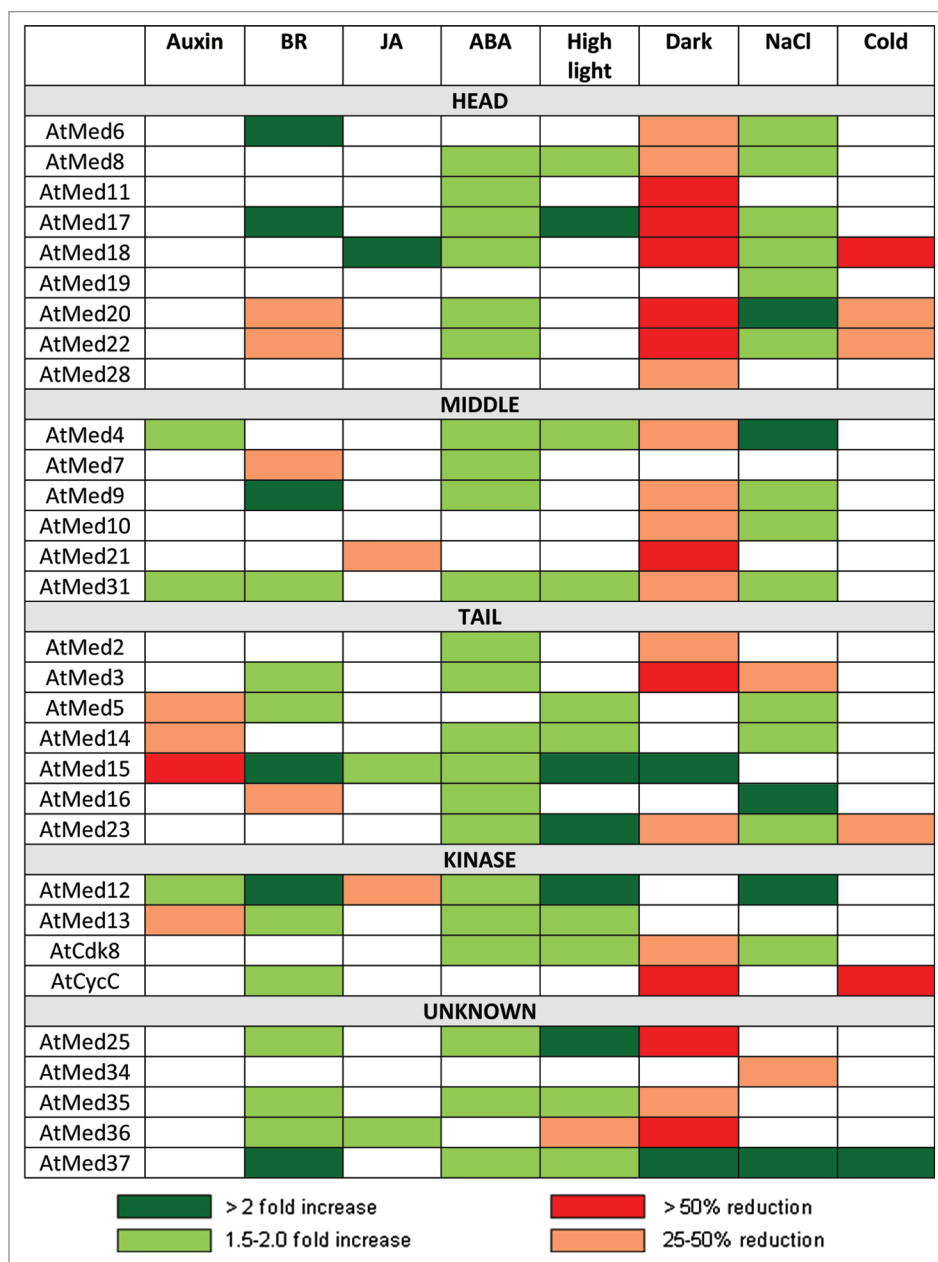No potential conflicts of interest were disclosed.

Figure 7. Summary of changes in the transcript abundance of *AtMed* genes in response to different hormones or stresses. Color legends explaining the range of fold changes are shown at the bottom.

Supplemental Material

Supplemental material may be downloaded here:
www.landesbioscience.com/journals/psb/article/22438/

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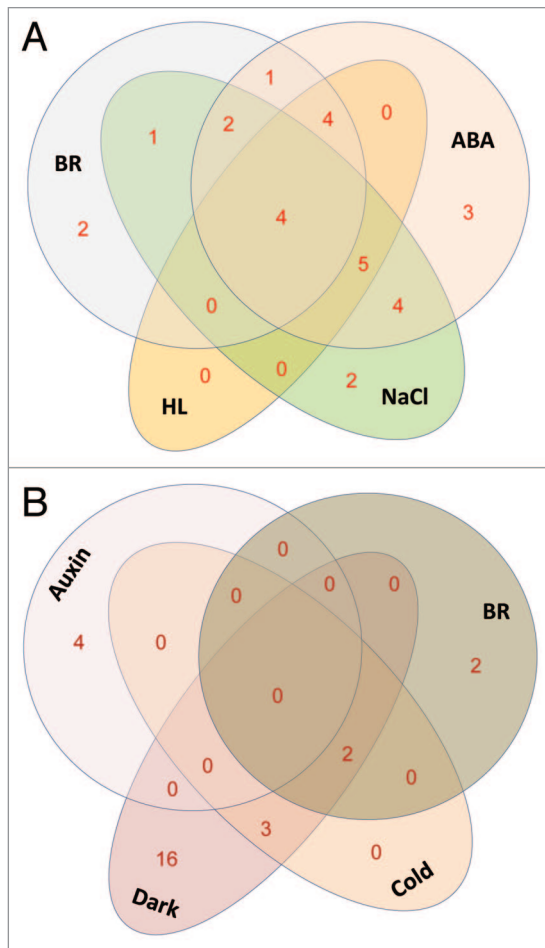
Figure 8. Schematic representation of number of *AtMed* genes commonly upregulated (A) or downregulated (B) by different factors (hormones or stresses) mentioned in bold black letters. BR, brassinosteroid; ABA, abscic acid; HL, high white light. Numerals denote number of *AtMed* genes.

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