

Handling calcium signaling: *Arabidopsis* CaMs and CMLs

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The *Arabidopsis* genome harbors seven calmodulin (CaM) and 50 CaM-like (CML) genes that encode potential calcium sensors. The CaMs encode only four protein isoforms. Selective pressure to maintain multiple CaMs indicates nonredundancy. Sequence divergence, even in the EF hand calcium-binding motif, exists among the CMLs and, therefore, divergent functions are likely to have evolved. Expression data recently available from Massively Parallel Signature Sequencing and Genevestigator compilation of microarrays are reviewed. The seven *Arabidopsis* CaMs are highly and relatively uniformly expressed. Differential expression is evident among the distinct CMLs over developmental stages, in various organs and in response to many different stimuli. In spite of the potential importance in mediating plant calcium signaling, the physiological functions of the *Arabidopsis* CaMs and CMLs remain largely unknown.

Calcium signaling and perception

Calcium ions (Ca^{2+}) at elevated levels can be toxic to cellular energy metabolism and, therefore, are pumped from the cytosol, leaving submicromolar resting levels. Capitalizing on the steep Ca^{2+} gradient across membranes facing the cytosol, cells use the gating of membrane Ca^{2+} channels and subsequent transient increases in cytosolic Ca^{2+} as signals. The value of such cytosolic Ca^{2+} fluctuations is apparent both in the ubiquity of Ca^{2+} signaling among diverse eukaryotes and the multiplicity of stimuli that result in cytosolic Ca^{2+} fluctuations. Indeed, so many diverse stimuli result in cytosolic Ca^{2+} increases in plant cells that a quick overview of the published literature can lead one to believe that nearly all environmental, hormonal and developmental stimuli use Ca^{2+} as a signaling molecule. The consequent dilemma is then blatantly apparent: how can diverse stimuli result in distinct and appropriate responses if all act through the common intermediary of Ca^{2+} ?

This question is crucially important to answer if we are to decipher the details of how plants perceive their developmental state and environmental conditions and mount appropriate responses.

There can be magnitudinal, spatial and temporal aspects of individual Ca^{2+} signals, as there are in animal cells, and these characteristics might be informative in ways that enable decoding of this 'Ca²⁺ signature' to elicit

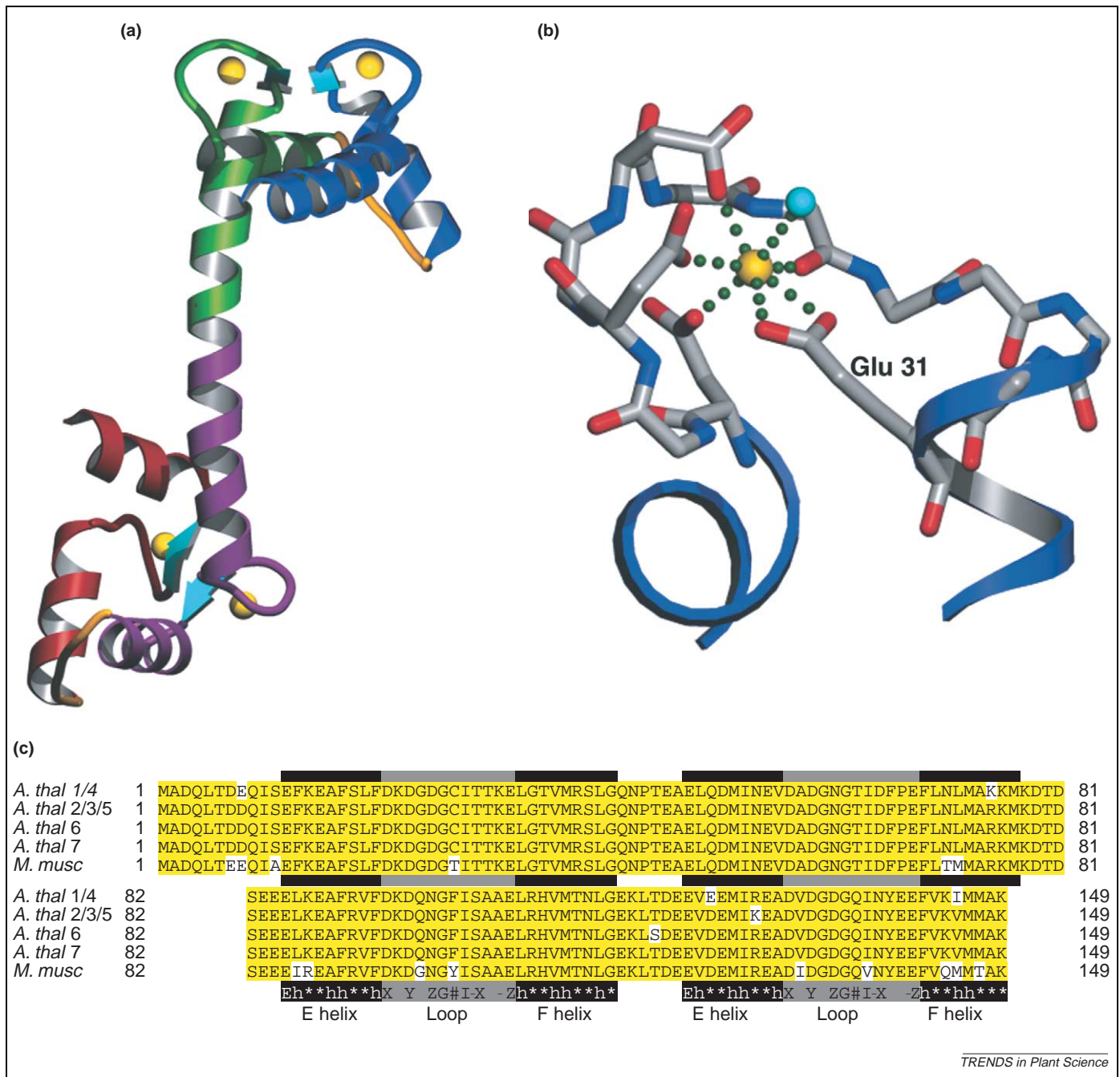
specific and appropriate responses [1–6]. Defining such Ca^{2+} signal characteristics and demonstrating the necessity of specific signal characteristics in inducing distinct responses is a laudable, if technically difficult, goal towards which to strive.

The other fundamental component to the mystery of Ca^{2+} signaling is the cellular machinery that interprets and specifically transduces this information into appropriate cellular responses. Enter calmodulin (CaM). CaM, ubiquitous among eukaryotes and essential for life, is composed of two pairs of Ca^{2+} binding sites joined by a linker domain [7] (Figure 1a). A binding site is composed of two helices, called the E and F helices, flanking a Ca^{2+} -binding loop in a structure that somewhat resembles a hand – hence the use of the term 'EF hand' for the Ca^{2+} -binding motif. CaM is a remarkable protein. It has no enzymatic activity. Its function resides, in part, in the ability to bind Ca^{2+} selectively with micromolar affinity and the consequent conformational changes that are induced upon Ca^{2+} binding. In addition, Ca^{2+} -induced conformational changes in CaM affect interactions with an abundance of target proteins and modulate target protein activity [7,8]. In this way, cytosolic Ca^{2+} changes perceived by CaM are transduced into altered target activity and, hence, a cellular response. Fully defining the function and mechanisms of CaM action is difficult because of the abundance and complexity of interaction partners. The conservation of CaM sequence in diverse eukaryotic cells probably reflects strict restrictions of structure required to maintain productive interactions with so many distinct targets.

The *Arabidopsis* genome sequence presents evidence that the problem of deciphering the complex functions of CaM is just the tip of the iceberg. Genomic mining reveals that there are ~232 EF hand-containing proteins encoded in the *Arabidopsis* genome [9], including seven genes that encode bona fide CaMs [10] (J. Braam *et al.*, unpublished) and 50 genes that encode CaM-like (CML) proteins composed almost entirely of CaM-like EF hand structures [10] (Supplementary material Figure S1). Perhaps these diverse and abundant potential Ca^{2+} sensors will provide new evidence about the specificities and mechanisms of plant Ca^{2+} signal transduction.

Here, we discuss insights obtainable from primary sequences and recently documented expression behaviors. Together these data begin to shed light on the likelihood that the CaMs and CMLs have taken on novel biochemical functions ('neofunctionalization')

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Figure 1. Calmodulin. (a) The structure of CaM showing the four helix-loop-helix Ca²⁺-binding sites in four different colors. The central Ca²⁺-binding loops are shown in cyan. Yellow spheres represent calcium ions. Adapted, with permission, from [26]. (b) Structure of the first Ca²⁺-binding loop of CaM. The side-chains that are typically involved in Ca²⁺ coordination are shown. The yellow spheres represent the calcium ions and the water solvent molecule is shown in cyan. Adapted, with permission, from [26]. (c) The four CaM isoforms encoded by the seven *Arabidopsis* CAM genes are aligned with the mouse CaM. Amino acid numbering is indicated on the left and right. Note that the initiator methionine (M) is probably removed from the mature protein such that the mature proteins are 148 amino acids long. The regions corresponding to the E helices, Ca²⁺-binding loops and F helices are indicated by the black, grey and black bars, respectively. The consensus sequences for these regions are indicated beneath the relevant sequences. 'E' stands for glutamic acid, 'h' for hydrophobic amino acid, '*' for any amino acid and X, Y, Z, G, #, I, -X -Z are defined in the text. Amino acid sequence identities are shaded in yellow. Portions of (c) are adapted, with permission, from [10].

and/or disparate gene expression behaviors ('subfunctionalization') that might explain the presence and maintenance of the large gene family in the *Arabidopsis* genome [10,11]. The potential of this protein family has just begun to be appreciated. Understanding the biochemical and cellular functions of the CaMs and CMLs might reveal how the versatile Ca²⁺ signal facilitates the dynamic behaviors and environmental adaptability seen in plants.

Seven *Arabidopsis* CAMs

There are seven *Arabidopsis* (Col-O) CAM genes encoding proteins that share (89% identity to vertebrate CaMs [10] (Figure 1c, Supplementary material Figure S1). The sequence variations are predicted to not alter function significantly, hence these proteins are considered to be true CaMs [10]. Intriguingly, the seven distinct genomic loci encode only four protein isoforms. The first isoform is encoded by *CAM1* and *CAM4*; *CAM2*, *CAM3* and *CAM5*

encode a second *Arabidopsis* CaM isoform. CaM1/CaM4 differ from CaM7 by four amino acids; CaM2/CaM3/CaM5 and CaM6 differ from CaM7 by only a single amino acid.

The primary structures of *Arabidopsis* CaMs are highly similar to those of animal CaMs in both the Ca²⁺-binding loops and E and F helices (Figure 1c). The canonical 12-amino acid loop of the CaM EF hand motif binds Ca²⁺ with a pentagonal bipyramidal geometry with seven coordination sites (Figure 1b) [12]. The first, third, fifth, seventh, ninth and twelfth amino acids (alternatively called X, Y, Z, #, -X, -Z) in the loop chelate Ca²⁺. Ca²⁺ is coordinated mostly through side chain oxygens, except for the participation of the main chain oxygen of the # amino acid and the frequent use of a hydrogen-bonded water molecule at position -X. The amino acids involved in chelation are strongly conserved. Position X is usually aspartate (D); Y is aspartate (D) or asparagine (N); Z is aspartate (D), asparagine (N), or serine (S); position # tolerates a variety of amino acids; -X also varies but is usually aspartate (D), asparagine (N), or serine (S); -Z, which contributes two coordination sites, is nearly always glutamate (E). Position six is also conserved as a glycine (G); G might enable the formation of a sharp turn within the loop. Position eight is usually isoleucine (I) and is thought to be important for hydrogen bond interactions with the paired EF loop. Plant CaMs commonly have a cysteine (C) in the first EF hand at position seven, although this is rare in nonplant CaMs. The flanking E and F helices are generally each nine amino acids long with a regular distribution of hydrophobic amino acids [12].

The four *Arabidopsis* CaM isoforms are highly similar; however, minor changes in CaMs might contribute to target specificity selection [13]. The *Arabidopsis* CaM isoforms have differential capacity to activate NAD kinase *in vitro* [14] and bind to kinesin-like motor protein and cyclic nucleotide-gated ion channels with differing affinities [15,16].

Based on sequence similarities to animal CaMs, it is probable that these plant CaMs share similar functions with animal CaMs. This conservation between plant and animal CaMs is not surprising because CaM is thought to be essential for eukaryotic cell function. The unexpected finding is that *Arabidopsis* has so many CaM-encoding genes. Furthermore, the presence and apparent selection to maintain multiple genes encoding identical or nearly identical proteins is particularly intriguing. One would expect that duplicated genes would gradually acquire mutations and diverge in sequence because of compensation provided by the closely related paralog.

Duplicated genes that fail to accumulate mutations must be under selective pressure to maintain the primary protein sequence. All the *Arabidopsis* CAM genes have pair-wise synonymous:nonsynonymous nucleotide ratios of between 41 and 255, calculated using SNAP (Synonymous/Non-synonymous Analysis Program, <http://www.hiv.lanl.gov/content/hiv-db/SNAP/WEBSNAP/SNAP.html>), where a value greater than one indicates positive selective pressure. Thus, there is apparently strong selection to maintain the primary amino acid sequences encoded by the seven CAM genes.

One possible explanation for the conservation of *Arabidopsis* CAM genes is that CaMs are needed at levels that exceed single gene output. Thus, simultaneous production from multiple genes is required. Alternatively, the different CAM genes might have evolved distinct expression patterns or regulatory behavior. Such subfunctionalization would enable selection for the retention of each paralog. As predicted for other proteins that have interacting protein partners [11], CaM mutations might result in dominant-negative effects and, thus, be strongly selected against. Indeed, a soybean CaM-like protein can competitively bind CaM targets and, therefore, might antagonize target activation by CaM [17]. However, *Arabidopsis* has many CaM-like proteins (see below); therefore, CaM-related sequences are tolerated in the genome and, thus, might not have high propensity to interfere with CaM function.

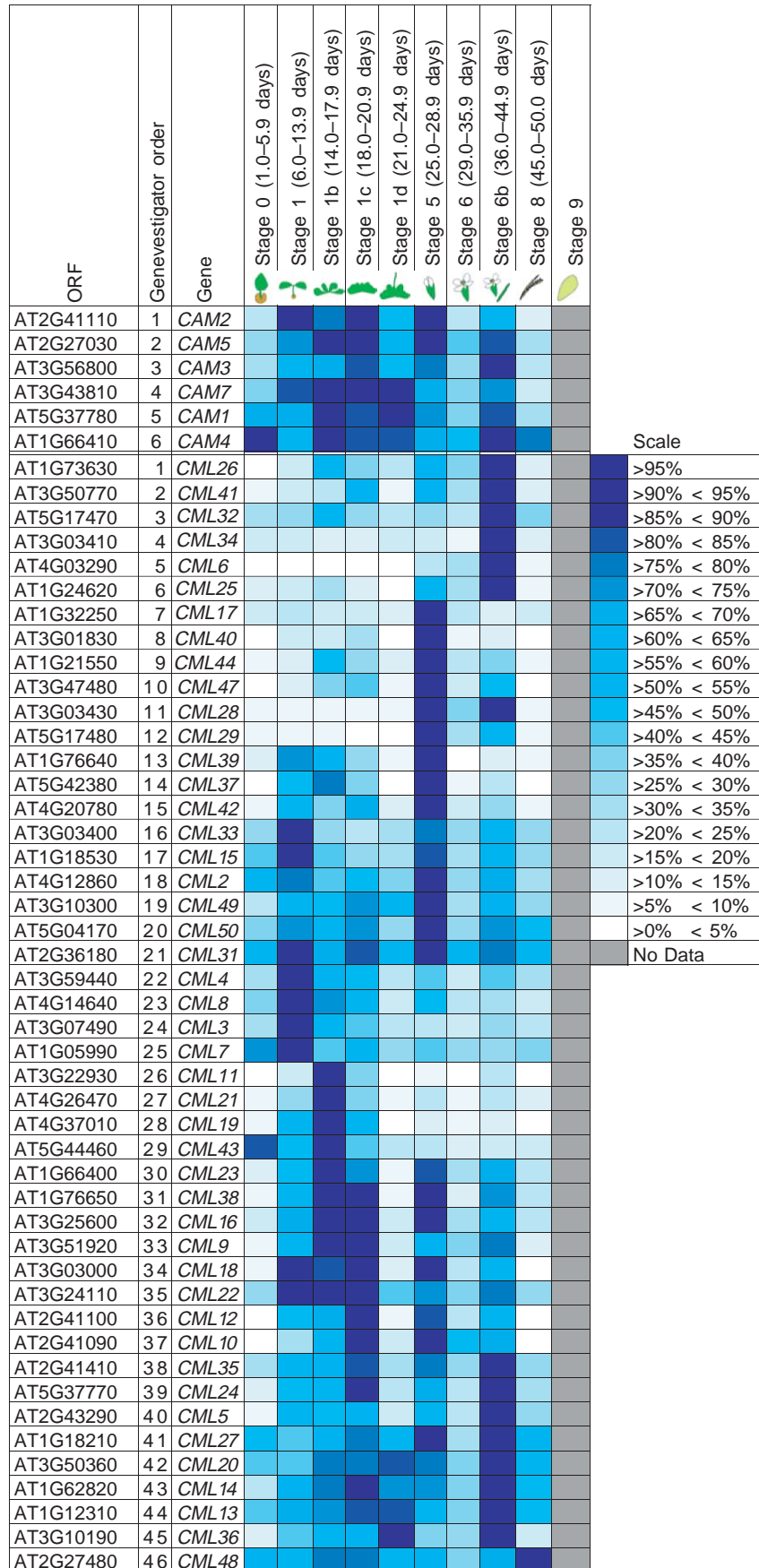
The CMLs: potential Ca²⁺ sensors

The 50-member CML family was identified as encoding proteins that are composed of EF hands and no other known or identifiable functional domains and share at least 16% amino acid identity with CaM [10]. Amino acid comparisons enable the separation of the CMLs into nine groups based on apparent divergence from CaM (see Supplementary material Figure S1). The CML family does not include proteins, such as the Ca²⁺-dependent protein kinases (CPKs) and calcineurin B-like proteins (CBLs), which have EF hand motifs and additional functional domains. All but one (CML1) of the CMLs have at least two identifiable EF hand-like motifs [10]. Most (31/50) CMLs have four predicted EF hands; one, CML12 (also known as TCH3), has six EF hands [10,18].

Although most of the EF hand motifs maintain the strict conservation of the Ca²⁺-coordinating residues, several CMLs have loop sequences that diverge from those of known CaMs [10]. For example, at least one hand of ten different CMLs has an E to D substitution in position 12 of the Ca²⁺-binding loop. This substitution might lower the binding affinity for Ca²⁺ and increase affinity for Mg²⁺ [19]. These and additional noncanonical amino acid substitutions in the generally conserved Ca²⁺-binding loop residues predict that six CMLs might have fewer than two sites capable of generating the pentagonal bipyramidal geometry for Ca²⁺ coordination. Although it is possible that these altered EF hand-like motifs no longer function in ion binding, it might be that at least some of these natural variants have evolved new structures that bind other cations or have distinct Ca²⁺-binding properties affecting on/off rates, affinities or the ability to undergo conformational changes.

Expression profiling of the *Arabidopsis* CAMs and CMLs

To gain insight into the potential subfunctionalization among the highly related CAMs and CMLs, we review the recently available expression data obtained by Massively Parallel Signature Sequencing (MPSS) [20] and compiled from ~1900 *Arabidopsis* Affymetrix microarray chip experiments as of February 2005 (<https://www.genevestigator.ethz.ch>) [21]. These techniques are capable of



differentiating among genes with as high a sequence identity as that shared by the *Arabidopsis* CAM genes [20,22].

MPSS identifies short unique gene sequences that are evidence of transcript presence in different plant organs [20]. Short sequences are derived from the most 3' site of a chosen restriction enzyme in cDNAs immobilized on individual beads. Immobilization permits hybridization-based sequencing of gene-identifying signature sequences from millions of cDNAs simultaneously. The abundance of a specific signature is representative of the transcript level present in the sample. These data are available in a searchable format online (<http://mpss.udel.edu/at/java.html>) and are summarized for seven CAMs and 42 CMLs in Supplementary material Table S1. Because MPSS relies on the presence of specific restriction enzyme sites within genes, values cannot be compared among genes. Thus, MPSS can be used to indicate transcript abundance differences for a given gene within different libraries but not to compare transcript abundances from different genes.

The Genevestigator online search tool Metaviewer (<https://www.genevestigator.ethz.ch>) [21] reports expression as a heat map where a color spectrum defines the relative expression of each gene. Microarray data are available for six CAMs and 46 CMLs. Genes are clustered based on related expression profiles during developmental stages, in specific organs, or following a stimulus (Figure 2 and Supplementary material Figures S2 and S3). Numerical values for transcript abundance are available in the Supplementary material Tables S2, S3 and S4.

The stages of plant development were based on age and are described briefly in the headings of Figure 2 and in detail on the Genevestigator website (<https://www.genevestigator.ethz.ch>). The CAMs are expressed during all developmental stages (Figure 2); the CAMs encoding identical proteins (CAM2/CAM3/CAM5 and CAM1/CAM4) are clustered together by Genevestigator, indicating that the closest paralogs share the most similar expression during development.

The CMLs cluster into five major groups (Figure 2). Expression from the first six genes peaks when seed formation begins. The second group, comprising nine genes, has highest expression at the early flowering stage. The next ten genes behave most similarly to the CAMs in that they are expressed at nearly all stages. The fourth gene cluster has the highest levels of expression during early developmental stages and shows a general decline in expression with aging; whereas the last group of nine genes behaves in the opposite fashion, having enhanced expression during later developmental stages.

CAM transcripts are associated with nearly every plant organ, whereas CML expression patterns cluster into five main groups (see Supplementary material Figure S2). The largest group of 13 CMLs shows expression in multiple organs, which is similar to the CAMs. CML11 and CML33 have highest expression in cell suspension. Eleven genes

are expressed almost exclusively in floral organs, with many having highest expression in the stamen. This finding is consistent with microarray data indicating CML2, CML6, CML21 and CML25 expression to be pollen specific [23,24]. A group of ten CMLs shows highest expression in leaves, cotyledons and sepals. The final group of ten genes has enriched expression in roots and radicles.

Genevestigator compilation data on stimulus-responsive CAM and CML expression clusters genes that behave similarly across many microarray experiments (see Supplementary material Figure S3). These compilations are derived from independent experiments that might have used distinct stimulus duration and application methods. Thus, heat maps in Supplementary material Figure S3 represent averages of behavior and are color coded by the average expression level change between stimulated and unstimulated conditions. Average fold change numerical values are included in Supplementary material Table S4.

The CAMs do not show strong transcript abundance changes in response to stimuli; by contrast, 35 CMLs have at least an averaged fivefold expression upregulation by at least one of the stimuli tested. CML37, CML39 and CML40 are among the most strongly regulated genes; the most potent stimuli affecting CML expression are treatments with cycloheximide, ozone, methyl jasmonate and salt stress. CML37 and CML40 expression increases 50-fold in cycloheximide-treated plants. Ozone results in at least 50-fold transcript abundance increases for CML37, CML39 and CML40. The expression levels of CML37 and CML39 are nearly 100- and 60-fold higher, respectively, in salt-stressed plants compared with plants that are not salt stressed, and CML39 is the gene most dramatically upregulated following methyl jasmonate treatment. Although Genevestigator data does not provide specific information about expression kinetics, it does cluster genes that behave similarly over many experiments.

Genes that occur in pairs of highly related paralogs [10] (Supplementary material Figure S1) more often show divergent expression characteristics than they do shared behaviors. A frequent observation is that one member of a pair has much higher expression than the other. Such expression magnitude differences are apparent between: CML4/CML5 (78.5% identity), CML15/CML16 (73.9% identity), CML19/CML20 (67.1% identity), CML26/CML27 (72.4% identity) and CML35/CML36 (53.6% identity). In addition, single members of a gene pair often show highly specialized expression characteristics. For example, CML15 expression is low and largely restricted to flower organs (Supplementary material Figure S2 and Supplementary material Tables S2, S3 and S4) and strongly induced by the elicitor syringolin (see Supplementary material Figure S3 and Supplementary material Table S4); whereas, CML16 transcripts that

Figure 2. Genevestigator *Arabidopsis* CAM and CML developmental stage expression. CAM and CML relative expression heat map obtained by searching results compiled from *Arabidopsis* microarray experiments. Genevestigator values and color coding were obtained in spreadsheet format by downloading and decoding the source code of the website with the assistance of VBA Macros. Dark-blue coloring represents highest expression as indicated by the scale. Genevestigator gene order is the result of clustering by expression similarity. The stages of development are indicated at the top by approximate days of growth and a depiction of the growth stage [21].

accumulate to levels >100-fold higher than those of *CML15* (Supplementary material Tables S2, S3 and S4) are found throughout most plant organs (Supplementary material Figure S2) and are relatively unaffected by the stimuli tested (see Supplementary material Figure S3). *CML20* expression occurs throughout many organs, whereas *CML19* expression is almost exclusively in the radicle and root (Supplementary material Figure S2). Furthermore, *CML19* expression is upregulated in response to methyl jasmonate, ozone, several bacterial pathogens, syringolin, the auxin transport inhibitor TIBA (2,3,5-triiodobenzoic acid) and senescence; *CML20* is only strongly induced (more than twofold) by syringolin (see Supplementary material Figure S3).

CML13/CML14 and *CML28/CML29* are pairs of highly related *CMLs* with 95% and 83% amino acid identity [10] (Supplementary material Figure S1), respectively. These gene pairs are similarly expressed during development, in organs and in response to stimuli (Figure 2, Supplementary material Figures S2 and S3). The expression similarities, in addition to their highly conserved primary structures, suggest that the duplication that gave rise to these gene pairs might have occurred relatively recently and mutation has not yet resulted in divergence in behavior or in the coding capacity of the progenitor gene. Alternatively, like the *CAMs*, selective pressure might be acting to maintain both the coding and expression information of these paralogous genes.

CAM and *CML* data obtained from the distinct techniques of EST identification (<http://www.Arabidopsis.org>), MPSS [20] and microarray analysis [21] generally yield similar findings. The EST data are less predictive than MPSS and Genevestigator are of localized expression, probably because the EST numbers are low. Genes, including *CML2*, *CML26*, *CML28* and *CML29*, with expression detection largely restricted to the inflorescence by MPSS (Supplementary material Table S1) also have expression increases during the transition from vegetative growth to flowering as assessed by Genevestigator (Figure 2). All the *CAMs* are shown by both MPSS and Genevestigator to be highly expressed in roots or radicles (see Supplementary material Figure S2 and Supplementary material Table S1). Both datasets also reveal that *CAM4* is also expressed in siliques or seeds (see Supplementary material Figure S2 and Supplementary material Table S1). Most genes shown by MPSS (Supplementary material Table S1) to have high root expression, such as *CML3*, *CML8*, *CML14*, *CML19*, *CML21*, *CML22* and *CML43*, are also identified as root or radicle-expressed genes by Genevestigator (see Supplementary material Figure S2). However, some data are contradictory. *CML7*, for example, is predicted to be expressed in many organs by Genevestigator but MPSS detects *CML7* transcripts only in roots. However, *CML7* has one of the lowest overall expression levels so these apparent discrepancies could be a consequence of detection sensitivity. *CML15* expression is detected at similar levels in all organs examined by MPSS but is reported by Genevestigator to be much higher in stamens than in other organs. The phenomena that lead to inconsistencies

in gene expression data need to be elucidated before these global analyses can be fully accepted.

Summary

The *Arabidopsis* genome has an abundance of *CAM* and *CAM*-like sequences. The apparent positive selection to maintain multiple copies of *CAM* genes, even those that encode identical isoforms, is intriguing. The recently available compiled expression data provide evidence for active expression of all seven *CAMs* and 50 *CMLs*. Thus, it is likely that none of these genes are pseudogenes and all function to generate RNA transcripts. The *CAMs* are not only highly related in their encoded proteins (Figure 1c) but also in their overall expression behaviors (Figure 2 and see Supplementary material Figures S2 and S3). Evidence of distinct organ or tissue expression might require more detailed cell- and/or tissue-specific analyses. One documented difference among the *CAMs* is that *CAM2*, also known as *TCH1*, is the only *CAM* whose expression is upregulated by touch [22,25]. Compared with, for example, the expression levels of most of the *CMLs*, the *CAM* transcript levels are high (Supplementary material Tables S2, S3 and S4). The high level of relatively uniform expression, which is comparable to *TUB4* encoding tubulin (J. Braam *et al.*, unpublished), might be an indication of a requirement for abundant quantities of CaM in plant cells. Reverse genetic experiments are currently one feasible approach to test the hypothesis that multiple *CAMs* are needed to provide sufficient CaM function. In addition, mutants might reveal potential differential and/or essential functions for the four different CaM isoforms and the multiple genes encoding identical protein isoforms.

The divergent *CML* family is likely to have evolved from progenitor *CAMs*. Unlike the *CAMs*, many of the *CMLs* appear to have undergone significant subfunctionalization. Several *CMLs* have expression limited to a small subset of organs and have dramatic expression changes in response to a variety of biotic and abiotic stimuli. Even the *CMLs* with closely related paralogs are generally found to display distinct expression characteristics. Thus, the different *CML* family members might have evolved in ways that enable their continued presence in the *Arabidopsis* genome to be under selective pressure. Determination of the biochemical and physiological functions of this large family of potential Ca²⁺ signal sensors remains an important challenge.

Acknowledgements

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Supplementary data

Supplementary material associated with this article can be found at [doi:10.1016/j.tplants.2005.07.001](https://doi.org/10.1016/j.tplants.2005.07.001)


- Figure S1: bootstrapped phylogenetic tree based on amino acid similarities separating the CaM/CML

family into nine groups. Adapted, with permission, from [10].

- Figure S2: Genevestigator *Arabidopsis CAM* and *CML* organ expression.
- Figure S3: Genevestigator *Arabidopsis CAM* and *CML* stimuli-induced expression.
- Table S1: MPSS data for the *CAMs* and *CMLs*.
- Table S2: Numerical values for Genevestigator data in Figure 2.
- Table S3: Numerical values for Genevestigator data in Supplementary material Figure S2.
- Table S4: Numerical values for Genevestigator data in Supplementary material Figure S3.

References

- Berridge, M.J. *et al.* (2000) The calcium entry pas de deux. *Science* 287, 1604–1605
- Rudd, J.J. and Franklin-Tong, V.E. (2001) Unravelling response-specificity in Ca²⁺ signalling pathways in plant cells. *New Phytol.* 151, 7–33
- Sanders, D. *et al.* (2002) Calcium at the crossroads of signaling. *Plant Cell* 14, S401–S417
- Ng, C.K-Y. and McAinsh, M.R. (2003) Encoding specificity in plant calcium signalling: hot-spotting the ups and downs and waves. *Ann. Bot. (Lond.)* 92, 477–485
- Allen, G.J. and Schroeder, J.I. (2001) Combining genetics and cell biology to crack the code of plant cell calcium signaling. *Sci. STKE* 102, RE13
- Fasano, J.M. *et al.* (2002) Ionic signaling in plant responses to gravity and touch. *J. Plant Growth Regul.* 21, 71–88
- Chin, D. and Means, A.R. (2000) Calmodulin: a prototypical calcium sensor. *Trends Cell Biol.* 10, 322–328
- Snedden, W.A. and Fromm, H. (2001) Calmodulin as a versatile calcium signal transducer in plants. *New Phytol.* 151, 35–66
- Day, I.S. *et al.* (2002) Analysis of EF-hand-containing proteins in *Arabidopsis*. *Genome Biol.* 3, RESEARCH0056
- McCormack, E. and Braam, J. (2003) Calmodulins and related potential calcium sensors of *Arabidopsis*. *New Phytol.* 159, 585–598
- Veitia, R.A. (2005) Paralogs in polyploids: one for all and all for one? *Plant Cell* 17, 4–11
- Kretsinger, R.H. (1998) Classification and evolution of EF-hand proteins. *Biometals* 11, 277–295
- Bhattacharya, S. *et al.* (2004) Target selectivity in EF-hand calcium binding proteins. *Biochim. Biophys. Acta* 1742, 69–79
- Liao, B. *et al.* (1996) Differential stimulation of NAD kinase and binding of peptide substrates by wild-type and mutant plant calmodulin isoforms. *Arch. Biochem. Biophys.* 327, 53–60
- Reddy, V.S. *et al.* (1999) Interaction of a kinesin-like protein with calmodulin isoforms from *Arabidopsis*. *J. Biol. Chem.* 274, 31727–31733
- Kohler, C. and Neuhaus, G. (2000) Characterisation of calmodulin binding to cyclic nucleotide-gated ion channels from *Arabidopsis thaliana*. *FEBS Lett.* 471, 133–136
- Lee, S.H. *et al.* (1999) Competitive binding of calmodulin isoforms to calmodulin-binding proteins: implication for the function of calmodulin isoforms in plants. *Biochim. Biophys. Acta* 1433, 56–67
- Sistrunk, M.L. *et al.* (1994) *Arabidopsis TCH3* encodes a novel Ca²⁺ binding protein and shows environmentally induced and tissue-specific regulation. *Plant Cell* 6, 1553–1565
- Cates, M.S. *et al.* (2002) Molecular mechanisms of calcium and magnesium binding to parvalbumin. *Biophys. J.* 82, 1133–1146
- Meyers, B.C. *et al.* (2004) Analysis of the transcriptional complexity of *Arabidopsis thaliana* by massively parallel signature sequencing. *Nat. Biotechnol.* 22, 1006–1011
- Zimmermann, P. *et al.* (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol.* 136, 2621–2632
- Lee, D. *et al.* (2005) Genome-wide identification of touch- and darkness-regulated *Arabidopsis* genes: a focus on calmodulin-like and *XTH* genes. *New Phytol.* 165, 429–444
- Honys, D. and Twell, D. (2003) Comparative analysis of the *Arabidopsis* pollen transcriptome. *Plant Physiol.* 132, 640–652
- Becker, J.D. *et al.* (2003) Transcriptional profiling of *Arabidopsis* tissues reveals the unique characteristics of the pollen transcriptome. *Plant Physiol.* 133, 713–725
- Braam, J. and Davis, R.W. (1990) Rain-, wind- and touch-induced expression of calmodulin and calmodulin-related genes in *Arabidopsis*. *Cell* 60, 357–364
- Grabarek, Z. (2005) Structure of a trapped intermediate of calmodulin: calcium regulation of EF-hand proteins from a new perspective. *J. Mol. Biol.* 346, 1351–1366



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Supplementary material

Handling calcium signaling: *Arabidopsis* CaMs and CMLs

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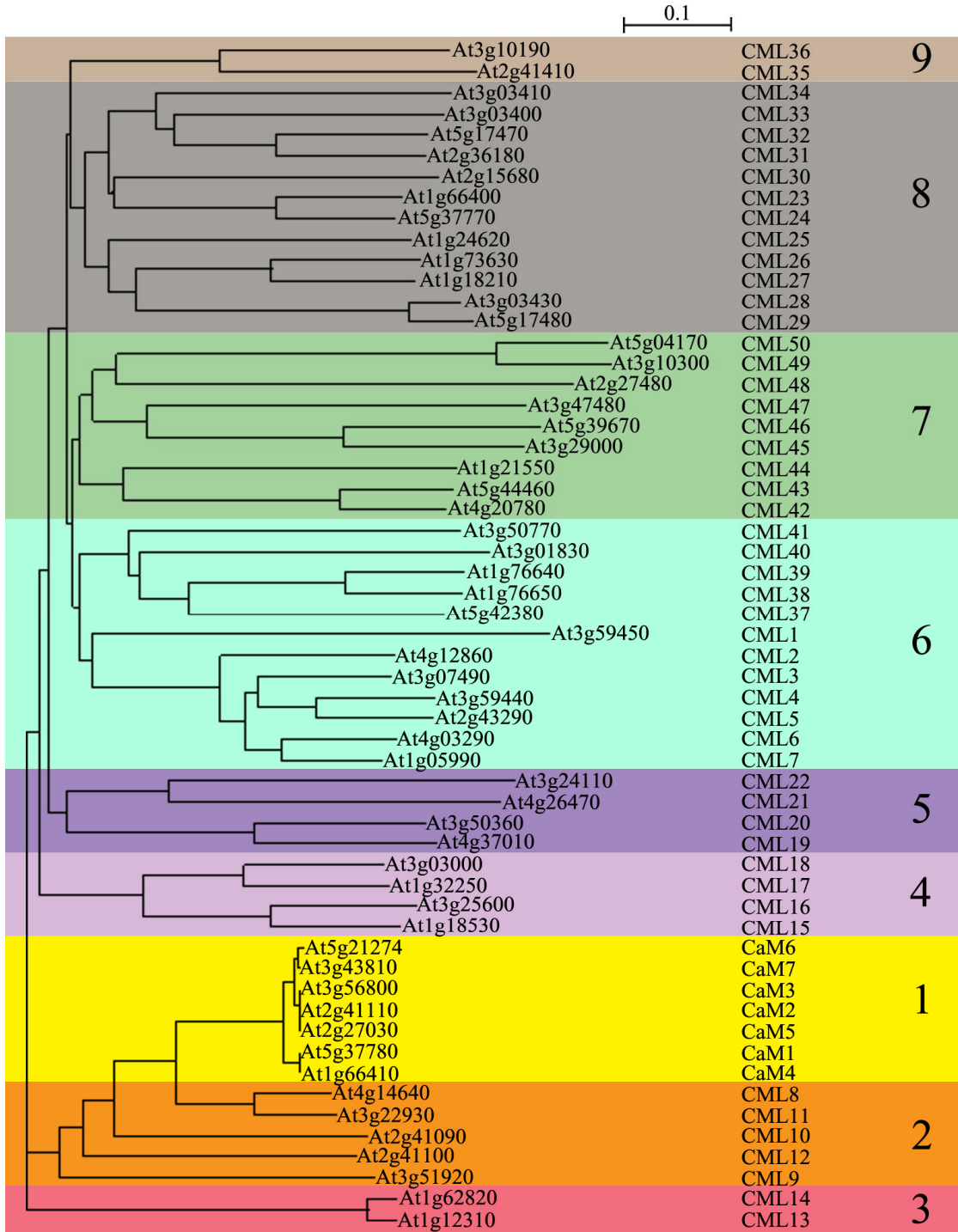


Figure S1. CaM and CML phylogenetic tree. Neighbor joining tree, based on amino acid similarities, separates 9 groups of CaMs and CMLs, as indicated by colors and numbers at right. The amino acid sequences of the predicted CaMs and CMLs were aligned and a bootstrapped phylogenetic tree with 1000 replicates was created (bootstrap values not shown). Both the open reading frame gene identifier numbers and CaM or CML names are shown. The distance indicated by '0.1' refers to the percent sequence divergence. Figure is adapted, with permission, from [1].

Reference

- 1 McCormack, E. and Braam, J. (2003) Calmodulins and related potential calcium sensors of *Arabidopsis*. *New Phytol.* 159, 585–598

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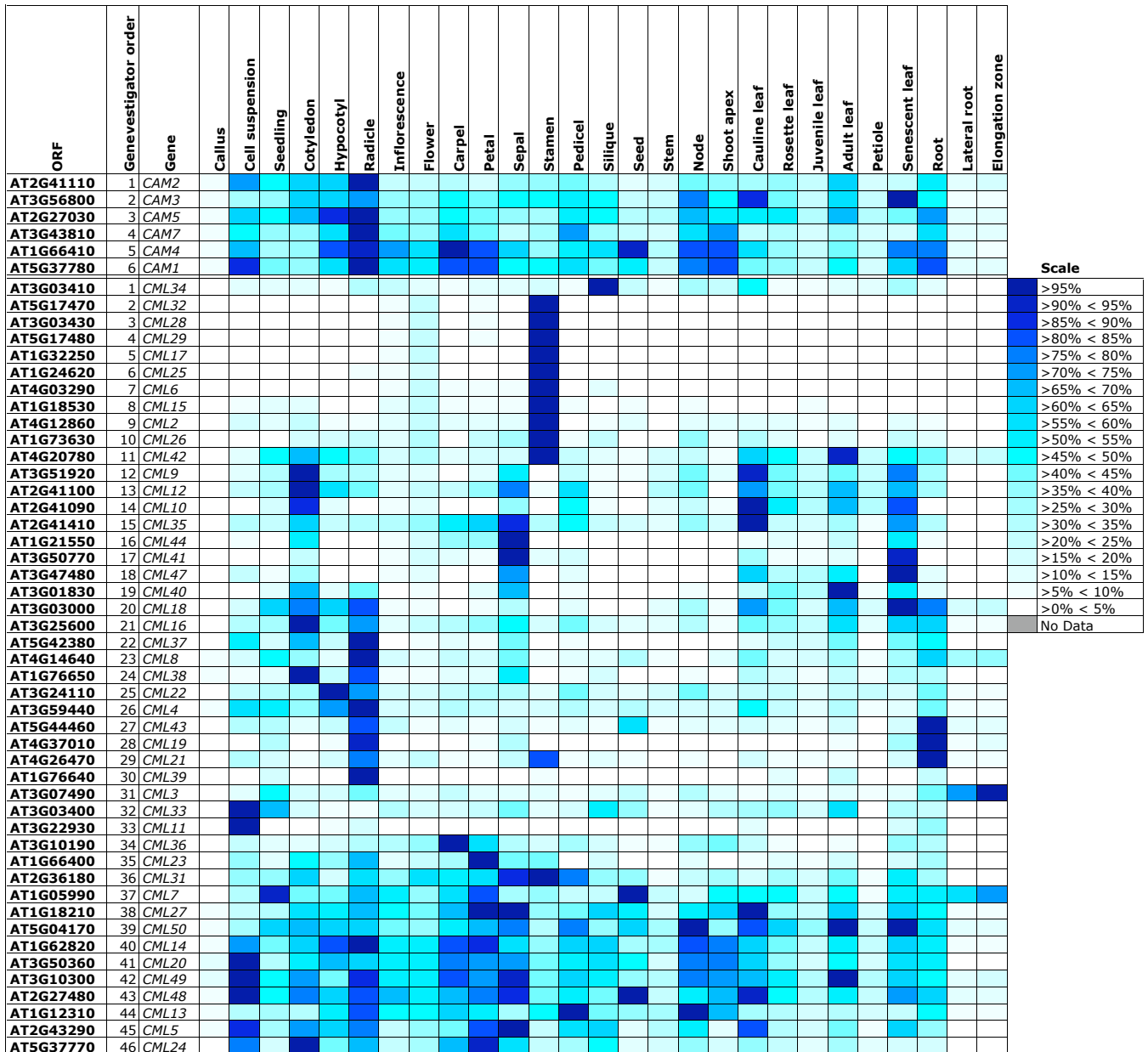
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Supplementary Figure S2. Genevestigator *Arabidopsis* CAM and CML organ expression. CAM and CML relative expression heat map obtained by searching results compiled from *Arabidopsis* microarray experiments. Genevestigator values, color coding and grouping were obtained as described for Figure 2. Plant organs are indicated at the top [1].

Reference

1 Zimmermann, P. et al. (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol.* 136, 2621–2632

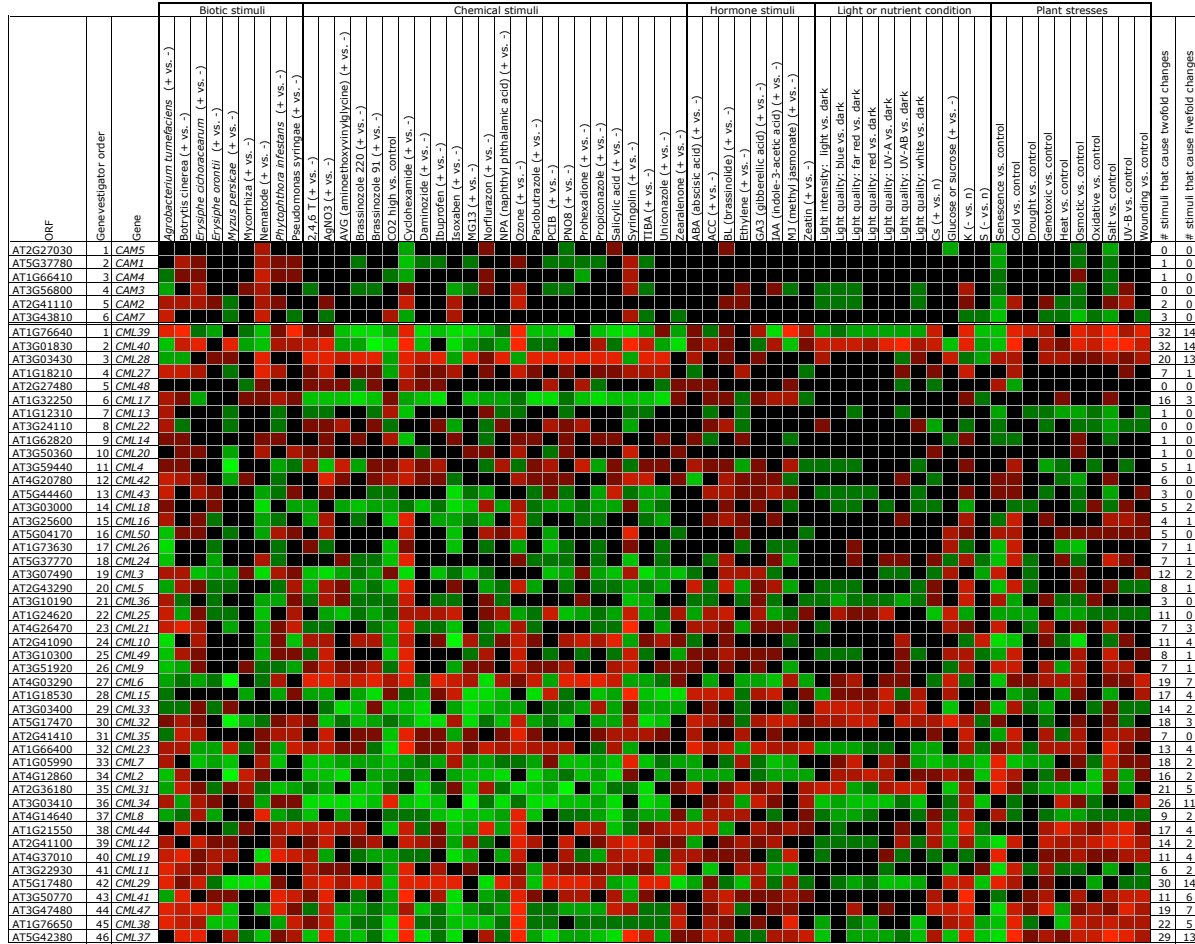


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Supplementary Figure S3. Genevestigator CAM and CML stimuli-induced expression. CAM and CML relative expression heat map obtained by searching results compiled from *Arabidopsis* microarray experiments. Genevestigator values, color coding and grouping were obtained as described for Figure 2. Conditions are listed above the heat map. Genevestigator output order is given at the left of the gene name. Red indicates a higher expression level in the presence of stimulus (listed as "+" or the condition before "versus"). Green indicates lower expression in stimulated conditions. Black indicates no change between conditions. For each gene, the two right columns indicate the number of conditions that lead to twofold or fivefold changes in averaged expression. Abbreviations: ACC, L-aminocyclopropane-1-carboxylic acid; MG13, carbobenzoxy-leuciny-leucine; PCB, p-chlorophenoxyisobutyric acid; PNO8, N-octyl-3-nitro-2,4,6-trihydroxybenzamide; 2,4,6T, 2,4,6-trichlorophenoxyacetic acid; TIBA, 2,3,5-triiodobenzoic acid.



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Supplementary Table S1. MPSS data for the CAMs and CMLs [1]

Gene	Callus	Germinating seedlings	Leaf	Inflorescence	Silique	Root	SA 4h	SA 52h
CAM1	945	28	342	279	608	1009	225	329
CAM2	216	26	195	81	153	533	0	20
CAM3	725	78	437	421	826	1099	95	402
CAM4	1067	138	223	346	769	542	169	205
CAM5	0	0	15	44	44	0	5	0
CAM6	486	89	247	93	448	693	73	30
CAM7	339	64	235	351	550	1136	32	52
CML1	35	0	0	0	5	3	0	0
CML2	0	0	0	11	0	0	0	0
CML3	0	0	2	0	0	17	0	0
CML4	86	0	0	0	0	2	0	0
CML5	123	5	24	9	99	21	0	0
CML6	0	0	0	0	0	0	0	0
CML7	0	0	0	0	0	6	0	13
CML8	23	0	4	0	0	33	0	0
CML9	114	35	165	6	0	67	31	46
CML10	0	4	2	2	0	0	2	71
CML11	87	0	2	3	0	62	0	0
CML12	3097	2	1464	28	205	36	30	1602
CML13	344	44	221	312	434	413	0	14
CML14	42	14	24	51	70	135	11	10
CML15	1	30	19	23	26	48	5	0
CML16	141	0	34	34	44	50	0	0
CML17	0	0	0	2	23	0	0	0
CML18	70	11	30	0	0	62	0	16
CML19	4	5	0	0	0	147	0	9
CML20	0	0	0	0	0	0	0	0
CML21	80	6	0	7	0	70	0	0
CML22	0	0	2	0	16	31	0	0
CML23	87	3	9	0	7	12	2	0
CML24	498	36	257	35	212	67	3	57
CML25	0	0	0	0	0	0	0	0
CML26	5	2	7	36	14	25	0	0
CML27	222	9	81	159	176	297	2	0
CML28	0	0	0	13	0	0	0	0
CML29	0	0	0	9	0	0	0	0
CML30	0	0	12	0	19	8	0	0
CML31	0	0	0	0	0	0	0	0
CML32	0	0	0	0	0	0	0	0
CML33	0	0	0	0	0	0	0	0
CML34	0	0	0	0	0	0	0	0
CML35	1110	24	292	331	300	350	2	78
CML36	50	0	14	196	184	171	1	0
CML37	30	0	39	0	28	21	2	1
CML38	28	0	10	0	0	18	0	0
CML39	0	0	0	0	0	0	0	0
CML40	6	0	3	0	0	0	0	18
CML41	19	0	12	1	19	11	0	2
CML42	9	42	46	15	16	124	0	33
CML43	116	0	0	0	0	2	0	0
CML44	0	0	1	3	0	13	0	6
CML45	11	0	14	0	0	2	0	0
CML46	99	0	0	0	0	0	0	1
CML47	249	0	4	0	0	7	0	15
CML48	30	6	9	7	17	8	18	15
CML49	285	4	63	142	89	84	32	114
CML50	10	26	34	7	87	40	3	36

Abbreviations: MPSS, Massively Parallel Signature Sequencing; SA, salicylic acid.

Reference

- 1 Meyers, B.C. *et al.* (2004) Analysis of the transcriptional complexity of *Arabidopsis thaliana* by massively parallel signature sequencing. *Nat. Biotechnol.* 22, 1006–1011

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Table S2. Numerical values for Genevestigator data in Figure 2

ORF	Genevestigator order	Gene	Stage 0 (1.0–5.9 days) 	Stage 1 (6.0–13.9 days) 	Stage 1b (14.0–17.9 days) 	Stage 1c (18.0–20.9 days) 	Stage 1d (21.0–24.9 days) 	Stage 5 (25.0–28.9 days) 	Stage 6 (29.0–35.9 days) 	Stage 6b (36.0–44.9 days) 	Stage 8 (45.0–50.0 days) 	Stage 9 
AT5G37780	5	<i>CAM1</i>	4,121	4,294	6,251	5,058	5,378	4,683	2,475	5,329	1,813	
AT2G41110	1	<i>CAM2</i>	562	2,263	1,985	2,251	1,128	2,495	549	1,489	357	
AT3G56800	3	<i>CAM3</i>	1,900	4,110	4,824	5,855	3,586	5,609	2,428	7,289	1,723	
AT1G66410	6	<i>CAM4</i>	6,207	3,563	6,352	5,232	5,249	4,401	3,044	6,347	4,800	
AT2G27030	2	<i>CAM5</i>	959	2,188	2,928	2,989	1,746	2,810	1,336	2,434	791	
AT3G43810	4	<i>CAM7</i>	1,997	4,054	5,008	4,614	4,771	3,391	1,815	3,533	912	
AT4G12860	18	<i>CML2</i>	46	69	39	40	33	88	30	59	22	
AT3G07490	24	<i>CML3</i>	39	131	69	55	27	31	24	41	28	
AT3G59440	22	<i>CML4</i>	42	150	93	73	36	62	30	61	41	
AT2G43290	40	<i>CML5</i>	192	1,367	1,150	1,556	378	1,430	518	2,415	728	
AT4G03290	5	<i>CML6</i>	16	16	13	19	13	113	148	499	29	
AT1G05990	25	<i>CML7</i>	38	53	23	29	16	22	18	17	19	
AT4G14640	23	<i>CML8</i>	27	73	53	37	13	36	16	21	14	
AT3G51920	33	<i>CML9</i>	403	3,272	6,179	6,351	1,131	4,040	2,380	4,900	788	
AT2G41090	37	<i>CML10</i>	161	4,143	7,890	15,504	2,355	15,142	7,532	10,557	293	
AT3G22930	26	<i>CML11</i>	22	99	529	188	17	48	23	117	15	
AT2G41100	36	<i>CML12</i>	364	6,714	10,142	14,667	1,276	12,093	3,177	7,981	397	
AT1G12310	44	<i>CML13</i>	1,741	2,733	3,005	3,203	3,326	2,236	1,436	4,021	1,902	
AT1G62820	43	<i>CML14</i>	343	901	1,124	1,250	1,056	1,088	515	1,468	722	
AT1G18530	17	<i>CML15</i>	8	19	8	6	5	16	6	10	7	
AT3G25600	32	<i>CML16</i>	386	1,430	2,072	1,996	390	1,908	585	1,149	496	
AT1G32250	7	<i>CML17</i>	8	11	8	9	6	52	10	7	8	
AT3G03000	34	<i>CML18</i>	29	337	280	315	40	303	82	177	8	
AT4G37010	28	<i>CML19</i>	50	444	750	393	26	95	42	90	29	
AT3G50360	42	<i>CML20</i>	1,044	1,020	1,834	1,857	1,885	1,758	843	2,337	1,375	
AT4G26470	27	<i>CML21</i>	43	218	720	255	46	164	66	167	89	
AT3G24110	35	<i>CML22</i>	58	158	183	177	82	131	73	143	62	
AT1G66400	30	<i>CML23</i>	48	207	439	318	39	369	125	290	107	
AT5G37770	39	<i>CML24</i>	277	1,072	1,324	2,294	525	1,574	544	2,125	681	
AT1G24620	6	<i>CML25</i>	28	46	62	30	12	138	63	248	15	
AT1G73630	1	<i>CML26</i>	52	222	746	576	291	905	526	1,454	186	

AT1G18210	41	<i>CML27</i>	2,955	2,845	3,881	4,996	3,768	5,564	1,713	6,380	3,792	
AT3G03430	11	<i>CML28</i>	28	42	44	40	28	494	178	477	39	
AT5G17480	12	<i>CML29</i>	18	15	17	11	8	288	75	153	16	
AT2G36180	21	<i>CML31</i>	14	25	17	20	14	25	15	20	14	
AT5G17470	3	<i>CML32</i>	37	39	64	38	31	40	26	125	45	
AT3G03400	16	<i>CML33</i>	15	49	16	11	13	37	16	28	16	
AT3G03410	4	<i>CML34</i>	8	10	7	7	9	8	4	51	7	
AT2G41410	38	<i>CML35</i>	2,681	5,129	5,970	8,074	2,848	7,221	3,017	9,607	3,097	
AT3G10190	45	<i>CML36</i>	150	476	642	686	1,002	409	375	1,082	218	
AT5G42380	14	<i>CML37</i>	19	278	461	235	26	587	41	123	22	
AT1G76650	31	<i>CML38</i>	88	636	1,188	1,159	96	1,064	153	877	253	
AT1G76640	13	<i>CML39</i>	18	123	104	59	14	171	9	23	12	
AT3G01830	8	<i>CML40</i>	11	386	419	608	26	2,407	167	308	22	
AT3G50770	2	<i>CML41</i>	109	252	278	836	115	842	371	1,335	138	
AT4G20780	15	<i>CML42</i>	183	1258	833	1461	258	2100	357	712	153	
AT5G44460	29	<i>CML43</i>	541	328	659	281	139	160	92	128	125	
AT1G21550	9	<i>CML44</i>	31	76	271	187	62	592	143	220	35	
AT3G47480	10	<i>CML47</i>	44	161	438	517	71	1,186	236	563	32	
AT2G27480	46	<i>CML48</i>	240	219	286	290	212	239	138	255	376	
AT3G10300	19	<i>CML49</i>	556	1,300	1,016	1,607	1,144	2,229	622	1,395	767	
AT5G04170	20	<i>CML50</i>	731	1,510	1,169	1,501	653	2,057	646	1,446	992	

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Supplementary Table S3. Numerical values for gene/estimator data in supplementary material Figure S2

ORF	Gene	Callus	Cell suspension	Seedling	Cotyledon	Hypocotyl	Radicle	Inflo-rescence	Flower	Carpel	Petal	Sepal	Stamen	Petalicel	Silique	Seed	Stem	Node	Shoot apex	Cauline leaf	Rosette leaf	Juvenile leaf	Adult leaf	Petiole	Senescent leaf	Root	Lateral root	Elongation zone
AT3G37380	CAM1	618	8190	3864	3352	5673	9477	5238	7994	4527	8600	4327	4302	5415	4118	4993	2176	7360	3418	4122	1709	4460	1967	5969	7944	964	1037	
AT2G41110	CAM2	230	3117	2747	2563	4236	1940	1032	1063	655	1555	1045	1755	1246	608	757	1728	1642	1539	1537	886	2662	756	912	2313	716	836	
AT3G56800	CAM3	609	3240	2043	6135	6307	7291	4032	5054	4541	3747	5021	5274	4790	2468	2448	2172	8129	9150	4327	5109	5867	2205	10174	4744	873	820	
AT1G56610	CAM4	781	6261	2322	3737	7653	8714	6759	5631	5922	7879	5934	2567	5141	5369	8946	2805	7862	7804	5597	3735	2014	4224	2313	7392	7470	1037	
AT2G70230	CAM5	302	2602	2005	2681	3737	4352	1688	1646	2067	1888	1683	1391	2308	2037	1272	1110	2062	2369	2391	2293	1157	2903	1243	1850	3053	458	
AT3G43810	CAMP	617	4726	3652	3409	5566	9658	3907	3815	5722	4370	2012	3123	6936	3187	2215	1624	5654	7195	1931	2848	1808	3251	1808	1588	5638	948	
AT4G12660	CML2	5	75	61	100	38	37	66	109	47	46	47	453	22	58	13	55	46	53	46	53	46	53	46	53	46	53	4
AT3G07490	CML3	8	23	109	43	37	99	34	31	56	31	56	31	56	31	56	31	56	31	56	31	56	31	56	31	56	31	4
AT3G39440	CML4	21	139	130	92	174	242	48	45	65	54	45	44	61	43	41	41	46	41	44	114	56	36	63	25	33	99	
AT5G42300	CML5	152	3112	1003	2717	2200	2764	885	1291	1475	2035	3612	751	2098	2210	1042	1873	487	966	658	1491	236	2181	1279	174	141	141	
AT4G02390	CML6	3	11	15	36	7	6	276	755	222	285	265	3577	5	473	26	6	18	15	20	16	8	18	10	30	11	1	
AT1G05990	CML7	1	14	45	21	21	23	46	19	27	39	16	11	48	10	9	22	22	22	22	11	23	5	26	28	28	25	
AT4G14640	CML8	7	23	63	48	17	138	20	18	31	22	55	11	13	32	7	6	15	25	21	15	25	21	15	45	77	42	
AT3G51920	CML9	157	1684	3139	11975	3707	3026	1255	1257	432	1295	6213	207	2735	921	627	2298	4898	1327	11265	5068	2783	4971	2741	9176	4135	342	
AT2G41090	CML10	2	121	4204	22492	3107	85	2191	2868	558	1074	9649	626	12872	2125	111	3528	6127	2410	26408	14295	6862	17994	7834	22025	217	11	
AT3G22330	CML11	53	2440	90	40	128	411	26	30	58	60	99	32	18	23	37	53	15	263	49	57	68	59	371	871	54	51	
AT2G41100	CML12	272	4819	6095	21935	12910	9097	7783	16490	5229	7783	16490	5229	13141	1500	297	6135	9669	1069	16307	9687	5911	14321	6429	15232	7131	515	
AT1G21110	CML13	383	2093	2444	1827	3673	3447	3467	4605	3897	2430	2366	7349	2939	2368	2498	7030	5028	2450	1808	1209	2045	1257	1492	3452	493	565	
AT1G02820	CML14	126	1493	838	1247	1686	2065	1081	1152	1715	1800	1215	805	1298	632	645	1763	1642	1275	879	443	1052	472	1327	1083	122	130	
AT1G18130	CML15	1	10	17	19	3	5	16	33	11	9	8	12	10	8	12	4	8	7	5	7	14	7	2	5	5	1	
AT3G26500	CML16	1466	1119	1311	3900	1586	2823	586	791	1079	1432	1774	689	1699	689	578	536	1129	358	1553	1405	829	2139	535	2465	2404	268	
AT1G32350	CML17	1	10	10	9	10	10	10	10	142	9	23	11	645	10	6	12	5	8	6	5	7	6	5	7	2	2	2
AT3G02000	CML18	14	79	307	384	302	391	33	25	12	48	129	15	64	13	16	69	159	41	356	206	78	338	84	491	378	79	
AT4G37010	CML19	4	39	380	58	71	1261	60	56	6	116	404	43	35	13	10	18	65	58	38	10	464	1396	126	126	163	126	
AT3G50360	CML20	377	3648	981	1855	2469	1972	1887	2840	2558	1495	1798	1887	2199	668	1188	2790	2196	1344	705	1666	606	2166	1798	143	140	100	
AT4G28470	CML21	33	304	205	83	176	897	123	252	162	277	938	102	143	72	41	89	45	207	93	77	112	147	17	216	1101	85	
AT2G41110	CML22	19	108	141	178	535	381	94	99	122	136	158	75	200	87	81	84	233	105	123	113	68	134	117	98	224	42	
AT1G56400	CML23	58	645	182	834	690	1188	201	437	591	1750	737	722	86	325	87	80	257	52	116	213	133	341	69	309	599	53	
AT3G37370	CML24	100	4082	959	5167	2306	2511	4040	1078	1585	2541	4040	2993	1117	1652	2362	510	950	1943	838	1968	1228	762	1721	687	977	1033	
AT1G24620	CML25	4	25	39	6	26	176	183	521	58	101	70	2682	19	118	36	4	5	13	20	12	5	12	2	9	113	25	
AT1G78330	CML26	9	94	214	958	1950	1320	683	1426	261	1091	1764	5380	332	1137	73	226	2117	408	1885	632	234	654	235	1265	691	115	
AT1G18130	CML27	417	2282	2675	6240	4568	4088	6222	9214	9308	3216	4013	5872	4975	1481	4999	5536	8951	3402	2120	5829	1658	5962	6428	718	480	480	
AT3G03430	CML28	13	37	39	18	30	35	600	1787	173	450	207	42	60	302	42	33	60	37	26	297	38	20	44	10	56	41	
AT5G17480	CML29	1	18	13	4	15	5	324	990	27	266	140	4264	3	84	26	10	3	11	11	11	5	12	3	15	17	1	
AT2G36180	CML30	1	21	22	24	8	33	22	32	29	33	48	55	41	19	21	5	13	17	10	18	10	22	5	28	18	1	
AT5G17470	CML31	10	57	35	42	47	32	61	75	49	44	25	192	22	158	59	18	37	41	59	34	17	28	11	75	65	4	
AT3G34600	CML32	1	63	42	12	5	5	16	10	12	14	25	7	11	32	25	3	6	16	22	25	10	37	3	18	14	1	
AT3G04110	CML34	1	6	9	7	4	17	5	6	9	5	6	8	9	4	58	12	4	18	13	27	4	3	7	20	4	7	
AT2G41410	CML35	804	5618	4864	13056	4823	1658	5339	7730	11351	13485	18112	5888	10051	5129	4272	2488	7455	4093	21089	5054	3942	7393	1923	15425	5811	781	
AT1G01190	CML36	136	843	422	480	790	1131	991	1472	3904	2247	1121	665	1277	753	216	296	1474	1593	903	211	138	220	181	1443	988	97	
AT5G42380	CML37	11	670	240	890	313	1335	44	83	135	70	534	19	81	24	29	28	55	19	190	292	232	602	61	547	618	15	
AT1G76650	CML38	449	253	564	4785	992	3912	255	323	360	504	2444	108	253	841	182	205	266	108	1245	779	396	1194	425	1317	1251	69	
AT1G76640	CML39	5	12	107	5	21	701	15	14	13	7	13	41	6	6	3	4	11	6	11	6	12	166	8	3	167	21	
AT3G01830	CML40	5	56	348	1765	287	1095	89	207	33	57	169	83	251	59	17	89	149	21	561	1114	696	2551	223	1351	269	3	
AT3G50770	CML41	16	87	268	1640	274	153	452	1069	801	511	1164	274	156	169	160	160	246	539	160	2240	539	410	942	6435	156	34	
AT4G03970	CML42	63	262	1162	1157	1037	676	276	276	367	472	2381	281	407	256	198	397	481	228	1512	1113	550	2146	534	1099	988	376	
AT3G44460	CML43	46	309	293	246	239	899	97	98	137	88	161	100	130	103	601	79	112	142	192	193	134	83	164	40	186	1075	
AT1G21350	CML44	10	121	70	1276	28	63	156	386	848	949	2409	66	91	32	47	27	48	66	152	350	178	585	22	1284	165	11	
AT5G47480	CML45	56	570	176	829	71	82	127	254	79	59	1810	96	293	56	51	45	66	45	1513	681	840	1275	53	2525	311	12	
AT2G74880	CML48	39	426	202	248	282	372	249	299	325	299	325	244	248	189	47	27	46	80	410	212	122	239	102	326	280	55	
AT3G10300	CML49	365	2383	1182	1649	947	2087	1187	1206	1979	1651	2223	975	1532	1218	918	717	1852	1670	1575	1257	713	2269	585	1489	1110	295	
AT5G44700	CML50	171</																										

Handling calcium signaling: *Arabidopsis* CaMs and CMLs

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Supplementary Table S4. Numerical values for Genevestigator data in supplementary Figure S3

ORF	Genevestigator order	Gene	Biotic stimuli																	Chemical stimuli																
			<i>Agrobacterium tumefaciens</i> (+ vs. -)	<i>Borytis cinerea</i> (+ vs. -)	<i>Erysiphe cichoracearum</i> (+ vs. -)	<i>Erysiphe orontii</i> (+ vs. -)	<i>Myzus persicae</i> (+ vs. -)	<i>Mycorrhiza</i> (+ vs. -)	Nematode (+ vs. -)	<i>Phytophthora infestans</i> (+ vs. -)	<i>Pseudomonas syringae</i> (+ vs. -)	2,4,6-T (2,4,6-trichlorobenzoic acid) (+ vs. -)	AgNO ₃ (+ vs. -)	AVG (aminooxyvinylglycine) (+ vs. -)	Brassicinole 220 (+ vs. -)	Brassinole 91 (+ vs. -)	CO ₂ high vs. control	Cyclohexamide (+ vs. -)	Daminozide (+ vs. -)	Ibuprofen (+ vs. -)	Isoxaben (+ vs. -)	MG13 (carbenoxoyl- <i>l</i> -leucyl- <i>l</i> -leucyl-DL-valine) (+ vs. -)	Norflurazon (+ vs. -)	NP4 (naphthylphthalamic acid) (+ vs. -)	Ozone (+ vs. -)	Paclobutrazole (+ vs. -)	PCIB (p-phenylisobutyric acid) (+ vs. -)	PNO3 (N-octyl-3-nitro- <i>l</i> -tryptophanbenzamide) (+ vs. -)	Prohexadione (+ vs. -)	Propiconazole (+ vs. -)	Salicic acid (+ vs. -)	Syringolin (+ vs. -)				
AT5G37780	2	CAM1	0.935	1.436	1.183	1.084	1.029	1.07	1.72	1.224	1.166	0.932	1.005	0.897	0.869	0.89	1.028	0.49	0.802	0.785	0.97	0.856	1.042	0.916	1.208	0.926	0.764	0.87	0.86	0.862	0.941	1.442				
AT2G41110	5	CAM2	1.706	1.441	1.418	1.233	0.806	1.033	1.885	1.205	1.069	0.89	1.376	1.074	0.98	0.957	0.97	2.154	0.955	0.894	1.582	0.933	1.112	0.996	2.659	1.087	0.969	0.931	0.962	0.918	0.982	1.131				
AT3G56800	4	CAM3	0.596	0.903	1.592	1.034	0.98	1.321	1.447	0.96	0.929	0.852	1.007	0.957	0.832	0.861	0.907	1.19	1.062	0.95	0.681	0.862	1.205	0.906	1.44	0.957	0.731	0.868	0.989	0.896	0.956	1.415				
AT1G66410	3	CAM4	0.843	1.263	1.373	0.949	1.036	1.122	2.486	1.201	1.241	1.114	1.027	1.085	0.957	1.02	0.867	0.507	0.904	0.968	1.018	1.011	1.171	1.209	1.061	1.069	0.874	0.941	0.682	1.032	0.993	1.68				
AT2G27030	1	CAM5	0.896	0.997	1.088	0.979	0.896	1.047	1.477	1.004	0.921	1.118	1.055	1.087	0.882	0.925	1.036	0.575	1.01	1.069	0.903	1.02	1.164	1.047	1.143	0.962	1.118	0.804	0.921	0.9	1.221	1.043				
AT3G43810	6	CAM7	2.212	1.144	0.914	1.006	0.867	1.143	2.686	0.909	0.84	0.985	0.867	1.006	1.005	0.9	1.847	0.518	0.971	0.932	1.791	1.027	1.052	0.955	1.007	0.984	1.015	0.983	0.944	0.954	1.034	1.517				
AT4G12860	34	CML2	0.315	1.557	0.937	1.059	0	2.236	1.388	0.947	0.989	0.499	0.396	0.24	0.892	0.568	0.603	0.8	0.646	0.966	0.667	0.468	1.047	0.136	0.88	0.238	0.682	0.767	0.466	0.579	0.206	1.187				
AT3G07490	19	CML3	1.452	1.706	0.4	0.661	0.675	0	1.224	0.063	1.634	1.207	0.297	0.132	0.702	0.528	1.352	0.214	1.097	0.367	0.856	0.266	0.856	0.89	1.133	1.138	0.615	0.672	1.159	0.565	0.335	1.941				
AT3G59440	11	CML4	1.187	1.383	0.994	1.056	0	0.902	0.981	0.557	0.744	1.937	0.464	2.034	0.558	1.32	1.285	3.013	1.311	1.462	1.051	0.946	0.721	1.53	0.832	1.184	1.537	0.872	2.987	0.672	1.276	0.735				
AT2G43290	20	CML5	0.702	1.445	1.199	0.717	0.859	0.91	0.978	0.786	1.486	0.686	1.508	1.326	0.642	0.73	0.433	9.772	1.053	0.981	1.154	0.914	0.655	0.83	2.531	0.702	0.936	0.755	1.061	0.712	0.797	0.513				
AT4G03290	27	CML6	0.609	0.834	0.506	0.808	0	1.101	0.842	1.419	1.148	7.272	5.136	2.298	3.877	4.558	1.835	7.086	0.727	4.343	2.037	1.591	0.164	1.991	1.348	2.249	0.502	6.743	4.79	5.608	2.163	0.664				
AT1G05990	33	CML7	1.889	0.962	0.428	0.694	0.373	0.93	1.576	0.471	0.901	0.473	0.433	0.651	0.703	0.504	0.284	0.544	0.826	0.513	1.36	0.551	0.682	0.793	0.714	0.565	1.244	0.298	0.423	0.697	0.322	5.776				
AT4G14640	37	CML8	0.521	0.735	1.698	0.616	0.908	1.067	9.583	0.662	0.774	0.336	0.983	0.704	1.11	0.907	0.789	0.929	0.726	0.357	0.189	0.389	0.287	0.61	4.225	0.355	1.02	0.84	0.853	0.614	0.234	0.87				
AT3G51920	26	CML9	0.403	0.702	1.328	0.989	1.101	1.152	0.795	0.845	0.628	1.227	2.635	1.41	1.255	1.432	0.742	8.276	1.109	1.131	0.527	1.212	1.252	1.071	2.739	1.283	1.222	1.484	1.144	1.107	1.606	2.16				
AT2G41090	24	CML10	0.084	0.975	1.745	0.941	1.043	0.998	5.994	1.266	0.389	1.471	1.698	0.973	1.43	1.436	0.585	3.257	0.925	1.329	0.023	1.479	0.846	1.327	3.863	1.208	1.015	1.484	2.056	1.491	5.238	0.61				
AT3G22930	41	CML11	14.556	1.489	1.38	1.448	0.882	0.988	1.97	0.931	0.902	1.531	1.226	1.216	1.115	1.064	0.668	7.239	0.935	1.75	1.079	1.287	0.886	1.053	1.201	0.593	1.48	1.277	1.219	1.334	1.711	1.388				
AT2G41100	39	CML12	1.995	1.049	2.314	1.37	1.121	0.936	1.092	1.804	0.892	1.274	3.302	1.001	1.234	1.526	1.07	3.749	1.662	1.166	0.501	1.471	0.754	1.131	11.228	1.413	0.958	2.134	0.979	1.406	2.622	4.182				
AT1G12310	7	CML13	1.689	1.033	1.018	1.009	0.838	1.143	1.003	0.787	0.986	0.837	0.796	1.092	0.893	0.822	1.527	0.465	1.113	0.991	0.945	0.889	1.76	0.852	0.814	0.981	0.879	0.797	0.926	0.892	0.925	0.865				
AT1G62820	9	CML14	1.198	1.369	0.938	0.93	0.886	1.036	1.293	1.164	1.148	0.967	1.389	1.121	1.269	0.974	1.515	0.413	1.132	0.981	1.305	1.044	0.729	0.917	1.293	1.169	0.901	1.2	0.976	1.202	1.212	0.979				
AT1G18530	28	CML15	0.75	1.038	0.966	1.056	0.882	0.658	1	0.536	1.174	0.609	0.585	1.023	0.643	0.218	1.353	0.775	0.694	1.269	2.286	0.209	0.2	0.345	1	0.133	2.45	0.788	1.004	0.405	0.656	16.377				
AT3G25600	15	CML16	1.434	0.938	1.2	0.736	0.995	0.96	0.695	0.851	0.919	0.684	1.581	1.07	0.735	0.9	0.472	7.765	0.883	0.509	0.698	0.832	0.641	0.981	2.115	0.76	0.876	0.887	0.808	0.822	0.9	1.122				
AT1G32250	6	CML17	1.667	1.299	0.522	0.943	0.913	1.257	1.187	1.509	1.284	0.269	0.281	0.418	0.244	0.371	1.171	0.66	0.298	0.25	0.943	0.383	0.519	0.694	0.795	0.232	0.117	0.77	0.354	0.144	0.271	0.371				
AT3G03000	14	CML18	1.261	1.115	1.048	1.286	0.986	0.986	0.072	1.12	0.705	0.62	0.893	0.659	0.486	0.637	0.62	0.719	0.491	0.712	1.114	0.356	1.861	0.531	1.925	0.772	0.637	0.572	0.78	0.704	0.524	1.154				
AT4G37010	40	CML19	2.843	9.323	1.253	1.772	1.466	0.976	0.198	4.372	3.926	1.494	0.635	0.986	0.803	0.672	0.686	0.779	0.723	1.077	0.164	0.65	0.891	0.68	2.291	0.97	1.069	1.412	0.868	0.705	1.659	2.616				
AT3G50360	10	CML20	0.909	1.285	1.204	0.965	0.701	0.934	1.499	1.065	1.441	1.2	1.41	1.031	0.963	1.04	1.019	1.068	0.867	1.129	1.129	1.15	1.304	1.054	1.686	0.925	0.844	1.195	0.94	1.033	1.06	2.166				
AT4G26470	23	CML21	2.429	6.764	1.715	0.981	0.679	0.872	6.24	1.882	2.35	0.839	0.865	0.916	1.052	0.755	0.528	6.416	0.772	0.836	0.261	1.113	0.737	0.629	1.824	0.721	0.871	0.913	0.938	0.779	0.774	23.307				
AT3G24110	8	CML22	1.515	0.842	0.898	1.079	0.779	1.019	1.054	0.731	0.925	1.217	1.382	1.667	1.116	1.374	0.762	0.979	1.124	1.268	0.593	1.138	0.757	1.178	1.109	0.907	1.528	1.164	1.429	1.037	1.088	0.687				
AT1G66400	32	CML23	1.591	1.621	0.596	0.609	2.011	0.848	1.309	0.56	2.524	1.793	2.063	1.354	1.281	0.862	1.444	35.64	1.256	1.229	2.801	1.323	2.052	1.425	2.141	1.467	1.459	1.77	1.134	0.782	1.646	0.63				
AT5G37770	18	CML24	0.497	1.088	0.918	0.53	0.978	0.932	1.664	0.716	0.973	0.879	1.022	1.293	0.792	0.776	0.444	9.223	0.897	1.036	1.098	1.116	0.904	0.876	1.407	0.781	0.83	0.767	0.984	0.745	0.908	0.458				
AT1G24620	22	CML25	2.077	0.613	1.255	0.791	0.824	1.085	0.308	1.102	1.486	1.047	0.503	0.458	0.866	0.535	0.755	1.549	1.585	1.798	3.63	1.44	1.224	1.481	1.924	0.814	0.741	2.239	0.274	0.6	0.863	3.431				
AT1G73630	17	CML26	0.162	0.945	0.946	0.802	0.928	1.064	1.034	0.767	0.89	1.193	0.824	1.065	0.893	0.929	0.453	1.346	1.038	0.986	0.35	1.047	0.848	1.014	0.65	0.978	0.446	0.729	0.966	0.735	0.884	1.163				
AT1G18210	4	CML27	2.3	2.234	1.486	1.089	0.864	1.106	5.991	1.588	1.136	1.183	2.852	1.09	1.316	1.184	0.626	2.262	1.163	1.27	1.564	1.056	1.292	2.807	1.183	1.055	1.297	1.111	1.331	1.405	1.512					
AT3G03430	3	CML28	0.556	0.599	0.87	1.186	1.173	1.09	4.735	0.972	1.095	5.045	6.707	3.4	5.481	6.76	0.683	11.719	2.066	4.274	0.702	9.014	1.198	6.446	0.684	7.053	8.94	6.02	12.976	4.824	11.981	0.643				
AT5G17480	42	CML29	11.2	1.289	3.125	0.735	0.158	0.248	0.2	1.328	1.146	2.039	6.038	1.566	10.132	2.528	0.251	9.419	10.94	10.338	4.083	0.873	2.177	7.791	1.737	0.915	8.447	5.571	6.737	1.56	0.552	0.296				
AT2G36180	35	CML31	0.69	2.19	1.395	0.75	1.25	1.493	0.727	0.443	0.633	0.769																								

TIBA (2,3,5-trihydrobenzoic acid) (+ vs. -)	Hormone stimuli										Light or nutrient condition										Plant stresses										
	Uniconazole (+ vs. -)	Zearalenone (+ vs. -)	ABA (abscisic acid) (+ vs. -)	ACC (1-amine-cyclopropane-1-carboxylic acid) (+ vs. -)	BL (brassinolide) (+ vs. -)	Ethylene (+ vs. -)	GA3 (gibberellic acid) (+ vs. -)	IAA (indole-3-acetic acid) (+ vs. -)	MJ (methyl jasmonate) (+ vs. -)	Zeatin (+ vs. -)	Light intensity: light vs. dark	Light quality: blue vs. dark	Light quality: far red vs. dark	Light quality: red vs. dark	Light quality: UV-A vs. dark	Light quality: UV-AB vs. dark	Light quality: white vs. dark	CS (+ vs. n)	Glucose or sucrose (+ vs. -)	K (+ vs. n)	ε (- vs. n)	Senescence vs. control	Cold vs. control	Drought vs. control	Genotoxic vs. control	Heat vs. control	Osmotic vs. control	Oxidative vs. control	Salt vs. control	UV-B vs. control	Wounding vs. control
0.787	0.973	0.939	0.943	1.025	0.975	1.062	0.953	0.904	0.998	0.919	1.045	1.076	0.973	1.1	0.956	0.951	1.021	0.969	0.985	0.884	0.955	0.619	1.033	0.903	1.03	0.947	0.847	0.9	0.715	0.986	0.922
0.886	0.973	0.882	0.956	1.078	1.167	1.2	1.064	1.072	0.97	0.987	0.827	0.859	0.827	0.929	0.988	1.053	0.852	0.982	1.005	1.272	0.957	0.505	1.593	0.93	1.28	0.8	0.75	0.956	1.363	1.722	1.033
0.813	1.034	0.915	0.973	0.894	1.026	1.178	1.079	1.248	0.859	1.132	0.81	0.833	0.81	0.955	1.043	1.036	0.823	0.998	0.947	1.846	0.958	0.85	1.116	1.072	0.997	1.063	0.822	0.979	0.858	1.173	1.019
0.892	0.972	0.911	1.137	1.061	0.977	1.067	1.055	0.938	0.978	0.934	1.038	1.081	1.009	1.026	1.049	0.97	1.017	1.057	1.094	1.034	0.942	0.812	1.076	1.005	1.047	0.986	1.238	0.973	0.854	1.05	1.002
0.951	0.878	0.975	1.001	1.128	1.239	1.081	1.138	1.028	1.037	0.959	1.043	1.031	1.082	1.124	1.044	1.057	1.082	1.023	0.7	0.984	1.004	0.605	0.911	0.958	1.032	1	0.714	1.004	0.703	1.022	0.982
0.807	1.024	0.834	0.914	0.977	0.997	1.03	1.003	1.086	0.948	0.934	0.943	0.954	0.904	0.974	0.943	0.898	0.922	1.004	1.063	0.777	0.861	0.404	0.895	0.964	0.834	0.796	0.5	0.859	0.627	0.983	0.791
1.013	0.585	1.13	0.669	0.639	0.643	0.98	0.743	0.509	0.51	0.625	1.124	1.585	1.711	2.016	1.793	1.034	1.345	1.302	1.551	1.351	0.405	4.396	1.674	0.969	0.725	1.32	0.947	0.481	1.484	0.701	1.186
0.215	0.571	0.322	0.737	1.004	1.549	1.901	1.476	0.844	0.998	0.926	0.955	1.142	0.937	1.018	0.848	0.72	0.912	0.866	1.589	0.96	0.587	2.418	1.002	0.858	1.003	1.072	1.207	0.957	1.112	0.871	1.177
1.204	1.23	1.071	1.848	1.305	1.265	0.661	1.352	1.014	1.499	0.796	0.823	0.833	0.789	0.928	1.132	1.045	0.837	1.126	1.142	1.24	1.068	0.957	1.808	0.898	0.66	0.774	0.971	0.798	1.088	0.713	1.023
0.826	0.669	1.132	2.107	0.781	2.18	0.679	1.219	1.574	0.619	0.952	0.866	0.845	0.795	0.918	1.016	1.002	0.854	1.278	0.871	2.671	1.026	0.323	2.686	0.794	1.015	1.023	1.202	0.96	1.396	0.763	0.844
0.628	0.773	1.238	1.112	0.623	0.898	0.514	0.622	0.387	1.504	0.508	1.052	1.286	1.98	0.905	1.209	1.275	0.929	0.723	3	0.819	0.992	0.467	1.294	1.172	1.523	1.411	1.607	0.934	1.326	1.321	2.739
0.419	0.74	0.67	0.54	0.988	0.821	1.857	0.793	0.325	0.68	0.917	1.018	1.364	2.033	0.895	1.472	1.406	0.794	0.268	0.666	0.669	0.464	6.455	0.465	0.584	0.639	1.111	1.194	0.851	3.739	1.336	1.356
0.615	0.694	1.188	0.662	1.484	1.636	1.425	0.892	0.778	0.877	1.178	0.78	0.668	0.59	0.849	1.116	0.924	0.764	1.244	0.762	1.665	0.99	1.704	0.788	0.904	0.734	0.644	0.604	0.709	0.844	0.788	0.773
1.142	1.164	1.55	1.194	1.062	1.231	1.105	1.219	1.134	0.59	0.906	1.025	0.987	0.975	1.014	1.003	1.204	1.004	1.009	0.71	1.467	1.002	2.353	2.278	1.005	1.71	0.584	1.418	1.039	1.64	1.541	0.96
1.323	1.705	1.358	0.769	1.046	1.297	1.12	1.002	1.015	0.803	0.925	1.001	0.981	0.991	0.944	0.999	1.585	1.012	0.979	1.132	0.768	2.016	0.061	0.306	0.942	1.221	0.755	0.218	0.881	0.712	1.157	0.901
1.273	0.671	2.8	1.129	0.448	0.959	1.442	1.453	0.668	0.6	1.015	1.077	0.99	0.907	0.93	0.75	0.958	0.961	0.976	0.557	1.121	1.237	0.23	3.995	0.769	1.454	0.731	0.727	0.991	0.648	0.902	0.811
2.617	1.377	1.216	0.66	1.13	1.349	1.868	1.155	1.659	0.953	0.926	0.802	0.747	0.742	0.848	1.056	1.528	0.788	1.616	0.584	1.811	1.629	0.234	2.919	1.142	2.256	1.504	1.937	2.1	4.948	5.166	1.653
0.74	0.915	0.995	0.973	0.826	1.079	0.721	1.055	0.94	0.973	0.945	0.97	0.877	0.853	0.956	0.922	0.864	0.952	0.876	0.897	0.996	1.004	0.521	0.93	0.819	0.832	0.657	0.563	0.833	0.59	0.925	0.824
0.887	1.282	1.071	1.205	0.863	0.975	1.237	0.966	1.093	0.795	0.876	0.937	0.976	0.918	1.026	1.032	1.025	0.947	1.045	0.855	1.289	1.093	0.743	0.741	0.958	1.024	0.991	1.389	1.078	0.844	1.097	0.978
0.266	0.141	0.113	2.042	2.2	2.007	0.765	1.836	3.079	1.142	1.363	0.653	1.228	1.288	1.368	2.04	0.797	1.738	1.1	1.563	1.075	1.053	0.875	0.655	0.801	1.044	0.686	1.216	1.041	0.627	0.897	1.478
0.83	0.666	1.039	0.983	1.309	1.754	1.338	1.14	1.214	0.874	0.892	1.036	0.925	0.992	1.004	1.035	1.215	1.047	1.088	0.921	1.722	0.919	0.772	2.15	0.932	1.344	0.927	1.064	1.092	1.434	1.688	1.154
0.998	0.128	1.333	1.044	1.339	1.336	0.814	1.281	1.461	1.765	0.825	1.075	1.029	0.982	0.937	1.09	0.897	1.006	1	1.036	1.236	0.832	1.068	1.113	1.023	0.943	1.3	1.116	1.005	1.17	0.984	1.053
0.573	0.772	0.994	0.88	0.956	1.353	1	1.176	1.063	0.805	1.122	0.91	0.807	0.758	0.908	0.734	0.89	0.855	0.772	0.962	0.703	1.108	1.105	0.729	0.821	0.953	0.947	0.824	1.169	0.841	1.156	1.097
2.124	0.833	1.479	1.515	1.291	1.289	1.356	0.888	0.797	2.863	0.928	0.84	1.068	1.043	1.042	1.17	0.823	0.879	0.765	1.132	0.754	0.951	12.476	0.872	0.963	1.201	1.263	1.458	1.223	1.287	1.421	1.48
1.04	1.04	1.156	1.021	0.983	0.873	1.144	0.955	1.001	0.905	0.934	1.002	0.999	0.997	1.081	1.028	1.109	1.052	1.25	0.895	1.036	1.015	1.001	1.061	1.023	1.099	1.096	1.327	1.058	1.009	1.138	0.956
1.028	0.633	1.032	1.919	1.49	1.319	0.529	1.193	1.347	1.162	1.045	0.806	0.986	1.106	1.024	0.957	1.013	0.905	0.957	1.538	1.31	0.978	2.731	0.94	0.87	1.499	0.929	1.216	0.922	1.755	1.08	0.92
1.103	1.07	1.127	1.105	1.372	1.547	0.722	0.959	0.987	0.947	0.953	1.295	1.095	1.034	1.034	1.064	0.975	1.345	0.705	1.138	0.765	1.045	0.778	0.92	0.991	0.856	1.027	0.8	0.854	0.603	0.777	0.853
1.046	0.981	1.142	0.614	1.752	2.81	0.687	2.112	1.887	1.772	2.891	0.589	0.929	0.573	0.756	1.058	0.996	0.655	0.621	1.065	1.64	0.733	1.352	1.805	0.745	1.568	1.475	1.42	0.979	6.345	1.301	1.045
0.946	0.778	0.944	1.045	0.841	1.886	0.999	1.068	1.365	0.648	1.126	1.059	1.12	1.162	1.108	1.259	1.288	1.057	1.227	0.775	1.86	0.822	0.475	2.115	0.748	1.041	1.301	0.791	0.933	2.176	1.205	1.006
0.632	0.723	1.5	1.529	1.592	2.332	1.065	1.578	0.682	0.729	1.159	1.067	1.679	1.19	1.227	2.317	1.048	1.145	0.491	2.5	0.59	0.898	0.646	0.651	0.546	0.939	0.605	0.587	0.71	0.75	0.769	0.979
1.095	0.847	1.058	0.967	0.932	0.999	0.725	1.059	0.843	0.869	1.057	0.951	1.125	1.156	1.129	1.01	1.005	1.058	0.981	1.7	0.863	0.927	0.4	2.816	1.133	1.148	0.567	0.424	0.942	0.987	1.108	1.024
1.682	1.476	1.045	0.839	1.003	0.963	1.572	1.001	1.113	0.947	0.851	0.938	0.916	0.916	1.007	1.06	1.402	0.972	1.229	0.993	2.858	0.942	0.912	1.654	0.933	0.939	0.873	1.101	0.981	1.084	1.24	1.043
7.002	4.532	0.934	1.523	1.095	1.285	1.088	1.298	0.991	1.068	1.091	1.754	1.121	0.995	0.981	0.883	0.787	1.326	0.922	2.136	0.744	0.624	1.883	1.762	1.002	1.546	1.587	1.305	1.315	1.588	1.29	1.518
7.288	5.902	0.233	1.612	1.295	0.856	0.5	1.915	0.737	1.713	1.078	0.998	0.757	0.546	0.923	0.454	0.463	0.674	0.957	2.054	2.152	0.455	5.706	1.671	1.12	1.176	0.935	1.103	0.705	1.121	0.776	0.758
0.067	0.934	1.291	2.311	1.04	1.3	2.444	1.272	1.721	0.96	1.838	2.955	2.62	0.6	0.825	1.647	2.663	2.349	0.873	0.415	1.426	0.901	5.727	1.296	0.725	0.576	0.548	0.473	0.399	1.344	0.554	0.943
0.249	0.917	0.977	2.294	1.773	1.285	0.774	1.847	1.209	2.21	1.209	1.795	1.741	2.891	2.372	0.99	2.241	1.93	1.579	4.674	1.474	0.866	1.206	1.096	0.897	0.836	0.974	1.384	1.1	1.189	0.788	1.115
0.662	0.666	0.662	0.89	1.325	1.01	0.862	1.03	0.971	0.8	1.043	2.039	2.124	2.366	1.932	2.862	1.377	2.333	0.898	0.471	0.958	1.228	0.324	1.721	0.752	0.688	1.049	0.949	0.74			