ISSN : 2348-8069 Int.J.Adv. Res.Biol.Sci.2014; 1(6):136-153

International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Effect of gibberellin cross talk with other phytohormones on cellular growth and mitosis to endoreduplication transition

Viorel Munteanu, Victor Gordeev, Rodica Martea and Maria Duca

University of the Academy of Sciences of Moldova, Chisinau, Republic of Moldova

*Corresponding author: *viorelmunteanu.md@gmail.com*

Abstract

Plant hormones are involved in controlling overlapping physiological, genetic and metabolic processes in such a way that the outcomes depend on specific combinations of their activity rather than on their independent action. Gibberellins (GAs) represent growth regulators with an important role in organ elongation, seed germination, seed and flower development. Despite recent advances in elucidating multiple elements of the GA signaling cascade, detailed mechanisms by which GA responses are elicited but also their interference with regulation by other phytohormones are far from being understood. Our analysis of publicly available microarray expression data for the *A. thaliana* weak GA biosynthesis mutant *ga1*-5 revealed a set of differentially expressed genes under gibberellin treatment, most of which were also responsive to other phytohormones demonstrating an extensive overlap. We confirmed the relevance of the identified genes in GA response and revealed their involvement in growth and development associated processes in a synergistic or antagonistic manner with other phytohormones. Thus, a series of genes were associated with cell wall remodeling and with generating the turgor pressure necessary for cell wall expansion. In addition, we found an association of our GA responsive genes with the process of transition from mitosis to endoreduplication which was integrated with current knowledge.

Keywords: gibberellin signaling, phytohormone cross talk, endoreduplication, mitosis

Introduction

Phytohormones play a critical role in plant signaling ensuring an adequate response to the changes in the external and internal environments in order to regulate growth and development in both normal and stress conditions. They are synthesized in small quantities in specific organs/tissues and control either synergistically or antagonistically diverse processes during the life cycle of the organism such as germination, growth, metabolic rates, morphogenesis of new structures, senescence, response to stimuli of the external environment etc. The dynamic conditions of the environment and, especially the stress conditions, alter the normal levels of phytohormones and this strongly reflects on the processes of signaling, gene expression which, in turn, alter the flow of physiological processes.

In the last decades the key components involved in signaling and biosynthesis of the most important phytohormones such as gibberellin (GA), Indole-3-acetic acid (auxin, IAA), brassinosteroids (BR), cytokinin (CK), jasmonate (JA), abscisic acid (ABA) and ethylene (ET) were identified (Shan et al., 2012; Singh and Jwa, 2013). Numerous physiological and molecular studies show that all plant hormones interact with one or more additional phytohormones (Kohli et al., 2013), but in spite of the available description of pairs of interacting phytohormones, the regulatory network probably has additional levels of complexity represented by interactions between multiple phytohormones to mediate specific physiological processes (Weiss and Ori, 2007). Gibberellins represent a family of tetracyclic diterpenoid phytohormones which stimulate growth and developmental transitions in plants. These have a decisive role in physiological processes such as seed germination (Koornneef and van der Veen, 1980; Seo et al., 2009; Yamauchi et al., 2004), promotion of stem

elongation, leaf growth by means of cell division and expansion (Alabadi et al., 2008; de Lucas et al., 2008; Feng et al., 2008; Gallego-Bartolome et al., 2011; Ogawa et al., 2003). Gibberellins also stimulate developmental transitions from meristematic to shoot growth, maturation of young leafs and, importantly, the transition from vegetative to generative growth and other important aspects of flower development such as floral induction, flowering time, morphogenesis of generative organs (Galinha et al., 2009; Yu et al., 2004). Besides its crucial biological importance, exploitation of the knowledge about the GA signaling pathway turned into a dramatic increase in crop yields, a particularity that has already been exploited in several species (Peng et al., 1999).

The gibberellin signaling network topologically and functionally is composed of three modules:

(i) GA biosynthesis and degradation module – among which key enzymes are represented by GA20ox and GA3ox involved in gibberellin biosynthesis by sequential conversion of GA12 to GA4 (Hedden and Phillips, 2000). At the same time, C19 bioactive gibberellins are deactivated as a means of regulation by the members of the GA2ox family (Griffiths et al., 2006; Rieu et al., 2008a; Rieu et al., 2008b; Yamaguchi, 2008; Zentella et al., 2007), five in the case of *Arabidopsis*;

(ii) signal perception module represented by GID1 receptors with 3 orthologs in *Arabidopsis: GID1A, GID1B* and *GID1c* (Griffiths et al., 2006; Nakajima et al., 2006), which, after binding GA, increase their affinity for DELLA proteins, leading to the formation of the GA-GID1-DELLA complex (Griffiths et al., 2006; Willige et al., 2007);

(iii) response module which involves recognition of DELLA proteins (GAI, RGA, RGL1, RGL2 and RGL3) by the SCF E3 ubiquitin-ligase complex via the F-box SLY1 protein (Lechner et al., 2006), which further leads to the ubiquitination and degradation of DELLA proteins by the 26S proteasome (Dill et al., 2004; Sasaki et al., 2003).

The 5 *Arabidopsis* DELLA proteins (GAI, RGA, RGL1, RGL2 and RGL3), whose degradation is the primary step of GA signaling, serve as global repressors of the responses to GA with specialized but partially overlapping functions (Ikeda et al., 2001; Peng et al., 1997; Silverstone et al., 2001; Wen and Chang, 2002). RGA and GAI for instance repress vegetative growth and floral induction (Dill and Sun, 2001; King et al., 2001); RGL2 inhibits seed germination (Lee et al., 2002); RGA, RGL1 and RGL2 together modulate floral development (Cheng et al., 2004; Tyler et al., 2004), while RGL3 contributes to plant fitness during stress imposed by the external environment (Achard et al., 2008; Wild et al., 2012).

To implement these effects DELLAs interact with and alter the activity of many regulatory proteins, for instance, they control hypocotyl elongation by interacting with the PHYTOCHROME INTERACTING FACTORS (PIFs) (de Lucas et al., 2008; Feng et al., 2008) and BRASSINAZOLE RESISTANT1 (BZR1) (Bai et al., 2012; Gallego-Bartolome et al., 2012), they control floral transition and fructification by interacting respectively with SQUAMOSA PROMOTER BINDING-LIKE (SPL) and ALCATRAZ (ALC) (Arnaud et al., 2010; Yu et al., 2012) and contribute to the plant defense responses by interacting with JAZ proteins (JASMONATE ZIM-DOMAIN) (Hou et al., 2010; Wild et al., 2012; Yang et al., 2012). Thus by means of the DELLA proteins gibberellins not only control the expression of a multitude of target genes functioning in different paths and processes but also implement the crosstalk with the signaling pathways of other phytohormones.

Gibberellins are known to interact with all phytohormones discovered so far. Thus, GA and ABA have antagonistic roles in the regulation of numerous developmental and stimulus response processes (Rogers and Rogers, 1992; Weiss and Ori, 2007). By contrast, auxins promote GA responses by promoting DELLA degradation and by activating GA biosynthetic genes (Weiss and Ori, 2007). The interaction between GA and ET include both negative and positive mutual regulation depending on tissue and signaling context (Achard et al., 2003). Also, numerous antagonistic effects on developmental processes and signaling are exerted by GA and CK (Greenboim-Wainberg et al., 2005; Jasinski et al., 2005). It is known that CK and GA also interact in a developmentally – dependent manner where the two phytohormones inhibit mutual production. Given the role jasmonates play in stress response, the hormonal signals of GA and JA antagonistically and synergistically regulate diverse aspects of plant growth, development, and defense (Peng, 2009; Qi et al., 2014; Yang et al., 2012). Finally, GA together with BR, a biotic and abiotic stress counteracting hormone, promote many similar developmental responses in plants, one of which is the control of cell elongation and regulation of plant growth (Bai et al., 2012; Li et al., 2012a).

In spite of the knowledge about the upstream elements involved in the GA signaling cascade or about specific and local processes mediated by this signaling, the complete pathways and mechanisms of GA action in plants are still far from being understood. At the same time, few strictly specific targets of GA action have been identified. This fact suggests that the GA signaling pathway is largely overlapping with other phytohormone signaling and requires a time-specific and signal-specific crosstalk in order to adjust ontogenetic responses as well as communication and adaptation to the environment. Significant efforts were undertaken so far to uncover and describe the molecular mechanism of GA interaction with other phytohormones.

The aim of this study was to identify differentially expressed genes under GA treatment and to assess the main biological processes associated with these genes taking also into account their synergistic or antagonistic regulation by other phytohormones.

Materials and Methods

The microarray expression data

The design of the experiment, a detailed description of the methods used as well as the entire set of raw data were retrieved from *The Arabidopsis Information Resource (TAIR)* (Lamesch et al., 2012), *www.arabidopsis.org/portals/expression/microarray/ATGenExpress.jsp* on *www.arabidopsis.org,* March 31, 2014. The experimental dataset is also available in the *GEO* database (Edgar et al., 2002) with the identifier *GSE39384* (Goda et al., 2008). The *ATH1* Genome Array chip contains more than 22,500 probe sets representing approximately 24,000 *Arabidopsis* genes which cover most of the genome. In the original experimental setup, 7 day *A. thaliana* seedlings were treated both with mocks and the following 7 compounds: 1-amino-cyclopropane-1-carboxylic acid – ethylene (ET) precursor; cytokinin – CK; methyl-jasmonate – jasmonic acid (JA) derivative; indole-3-acetic acid – auxin (IAA); abscisic acid – ABA; gibberellic acid 3 – GA3; brassinolide - one of the brassinosteroid (BR) varieties. To assess the effect of gibberellin treatment, expression data from the weak GA biosynthesis mutant *ga1-5* were preferred over the wild type (Col1) expression data due to a low signal in the last case. For other phytohormones, data derived from wild type *Arabidopsis* plants were used.

Data preparation and identification of differentially expressed genes

Reading the raw data, their quality assessment and identification of the lists of differentially expressed genes were performed in the *R* programming environment (*R* version 3.1.0 available at *www.r-project.org*). Reading the cell files, quality checks, background correction and *Robust Multiarray Averaging* normalization (Irizarry et al., 2003) were carried with the *affy* (Gautier et al., 2004) and *simpleaffy* (Wilson and Miller, 2005) packages from the *BioConductor* resource. Differentially expressed genes between the hormone-treated and the mock-treated samples were obtained for each timepoint (0,5 h, 1h, 3h) and each phytohormone using linear modeling implemented by the *limma* package (Smyth, 2005) with parameters for absolute fold change = 1.5; and for p-value = 0.05. Annotation of Affymetrix gene ID's was carried with the *ath1121501.db* package (Carlson, 2013).

Visual representation of the gene-phytohormone interaction network was performed with the help of *Cytoscape* (Shannon et al., 2003).Functional annotation of genes with Gene Ontology (Ashburner et al., 2000) terms was performed in DAVID (*Database for Annotation, Visualization and Integrated Discovery*) (Huang da et al., 2009a, b).

Results and Discussion

Overlap of GA responses with those of other phytohormones

According to our data, from the total list of 107 genes differentially expressed after GA treatment only 32 were affected by gibberellin alone and 75 by at least a second phytohormone confirming extensive overlap of gibberellin responses with those of other phytohormones (figure 1). Further analysis has revealed the largest shared groups of differentially expressed genes with abscisic acid, jasmonate and auxin with 52, 38 and 31 genes respectively and significantly lower numbers for other phytohormones. Figure 1 shows synergistic and antagonistic relationships of gibberellin with other phytohormones and highlights the following trends: GA_3 downregulated genes are upregulated mainly by ABA and JA (25 and 19 genes respectively) and to a lesser extent by IAA (10 genes) but at the same time GA_3 on one hand and ABA, JA and IAA on the other hand share a set of commonly downregulated genes (16, 14 and 13 genes).

The network in figure 2 represents the association between the GA_3 responsive genes and all other phytohormones that affect their expression (the genes that responded to GA_3 only are not represented in the network).

The size of the nodes is proportional to the number of connections; the nodes are colored blue ifthe genes were downregulated after GA_3 treatment and red if they were upregulated

Analysis of gibberellin responsive genes and their relationship with other phytohormones

Our results reveal 107 differentially expressed genes under GA_3 treatment: 80 upregulated and 28 downregulated (supplementary table 1). Results of *Gene Ontology* assignment for the *Molecular Function* category are represented in table 1. We distinguish several genes involved in GA metabolism and signaling, a group of genes involved in growth-associated cell wall remodeling and, finally, a group of genes potentially involved in regulating turgor pressure (see the next chapters of Results and Discussion). All mentioned processes are related to developmental programs, especially to growth and cell expansion processes.

Genes involved in GA metabolism and signaling

In the list of differentially expressed genes several members involved in gibberellin signaling and metabolism were identified (table 2). As expected, following exogenous treatment, genes for GA catabolism (*GA2ox2*, *GA2ox6*) were upregulated, while genes for GA biosynthesis (*GA3ox1*, *GA20ox1*, *GA20ox2*) were downregulated. Besides this, two GA receptor genes *GID1A* and *GID1B* (orthologs to the rice single receptor *GID1*) were downregulated, presumably to further diminish the GA signal intensity. G*AI* – one of the DELLA major negative regulators of GA responses was upregulated (most of the protein is expected to be degraded after gibberellin administration) while the F-box protein SLY1 (Sleepy1) involved in targeting DELLA proteins for degradation was downregulated. Finally, the list of downregulated genes also includes SCL3 (Scarecrow-like 3) whose expression is known to be induced by DELLA and repressed by GA. It also seems to behave as an attenuator of DELLA proteins acting antagonistically with it in controlling both downstream GA responses and upstream GA biosynthetic genes (Zhang et al., 2011).

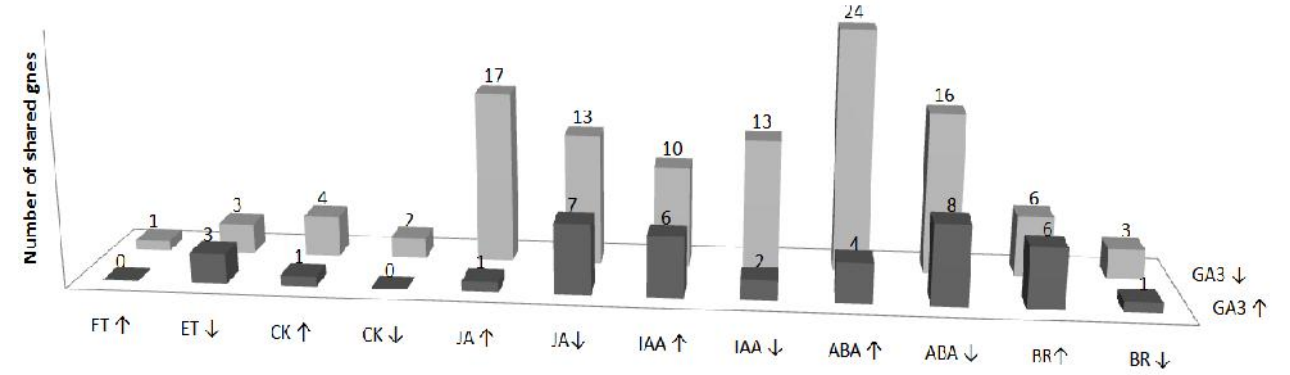
Most of the enumerated genes were detected at 1 h after GA_3 treatment except for *GA2ox2* and *GA20OX1* which were detected only after 3 h. This is probably indicative of sequential levels of regulation/ adaptation triggered by a prolonged hormonal signal.

An analysis of the genes listed above clearly indicates: first – that the analysis of microarray expression data has indeed generated a set of genes representative for a response to gibberellin, second – the direction in which the expression levels are affected clearly indicates the multiple methods the plant uses to implement negative feedback and to adapt to excessive GA signaling, third – the effect on gene expression varies from hormone to hormone.

ABA was found to stimulate the GA breakdown gene *GA2ox6*, consistent with its antagonistic role on GA signaling, but acted differently on the two GID1 receptors (table 2). The different role that the evolutionary diverged GID1B and GID1AC receptors play during seed germination has been the focus of a recent study (Voegele et al., 2011). The expression pattern of molecular factors involved in growth-associated cell wall remodeling during early germination suggested distinct roles for the GID1 receptors in endosperm cells. In agreement with this, our data shows differential control of these receptors by ABA at later stages of development, namely in 7 day-old seedlings (table 2).

Auxin acted repressively on GA signal transduction by both donwregulating the *GID1A* receptor gene and upregulating *GA2ox6* - one of the GA breakdown genes, but, at the same time, it upregulated two GA biosynthesis genes while downregulating one of them. Auxins are known to stimulate gibberellin biosynthesis (Frigerio et al., 2006; Valdovinos et al., 1967) and the two phytohormones are known to cooperate in a series of biological processes (Farquharson, 2010; Richter et al., 2013; Van Huizen et al., 1996). Such kind of behavior is probably a result of both the synergistic effect the two hormones have on growth and the different mechanisms in which these hormones act.

Finally, although jasmonate positively regulated the expression levels of the GA receptors it acted negatively on GA biosynthesis. Indeed, there is evidence that high levels of jasmonic acid was shown to downregulate key GA biosynthesis genes (Heinrich et al., 2013) which is also in line with the antagonistic effects of these hormones.



	ET ↑	ET ↓	CK ↑	CK ↓	JA ↑	JA↓	IAA ↑	IAA ↓	ABA ↑	ABA ↓	BR↑	BR ↓
GA3 ↑	0	3	1	0	1	7	6	2	4	8	6	1
GA3 ↓	1	3	4	2	17	13	10	13	24	16	6	3

Figure 1. Differentially expressed genes shared by gibberellins and other phytohormones:
↑ indicates upregulated genes while↓ indicates downregulated genes

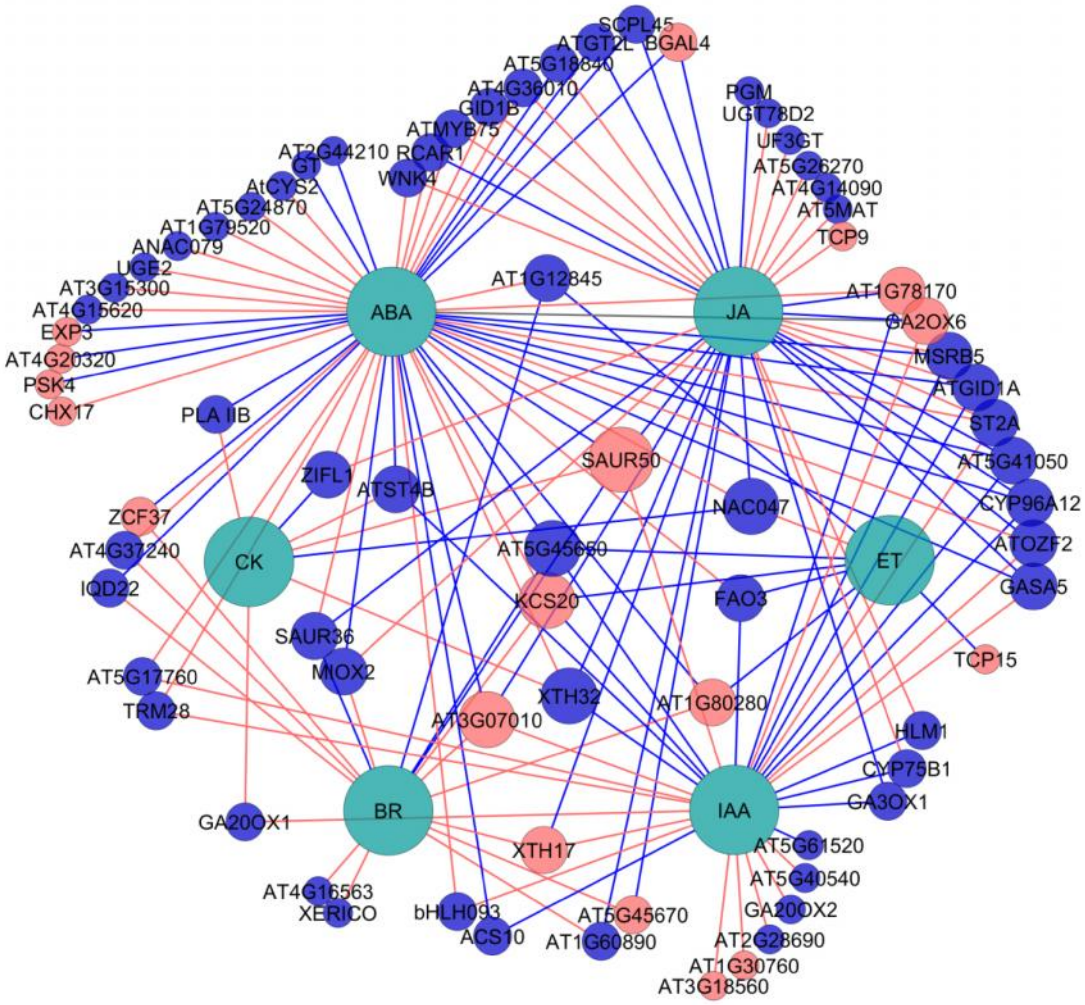


Figure 2. GA_3 responsive genes which changed expression levels also under treatment with other phytohormones.
The size of the nodes is proportional to the number of connections; the nodes are colored blue ifthe genes were downregulated after GA_3 treatment and red if they were upregulated

Table 1. GO Molecular Function of GA responsive genes

Molecular function	Nr.	GA_3 treatment effect	
		Upregulation	Downregulation
Catalytic activity - 24 genes			
transferase	11	-	*ATST4B, AtSTS, AT5MAT, ACS10, UGT78D2, UGT73C1, XTH32, GT, AT4G14090, AT3G07010, AT5G36160*
peptidase	3	-	*SCPL45, AT5G45650, AT4G16563*
carboxylesterase	6	*AT5G45670*	*AtCXE5, ESM1, AT1G28610, AT2G31100, AT5G38610*
ligase	1	*AT4G20320*	-
oxidoreductase	1	-	*MSRB5*
polygalacturonase	2	*JP630, ATXTH17*	-
Binding activity – 23 genes			
nucleotide binding	9	*AT1G30760*	*WNK4, HLM1, ATFAO3, XF1, ABCG, AT5G40540, AT1G60890, AT5G17760*
cofactor binding	1	-	*UGE2*
ion binding	10	*BGAL4, AT3G07200, AT3G07010*	*XERICO, CYP96A12, CYP75B1, GA5, MIOX2, PGM, PLDGAMMA2, AT5G24870*
lipid binding	1	-	*RCAR1*
calmodulin binding	1	-	*IQD22*
SNARE binding	1	*GOS11*	-
transport activity	6	*ADP1, ATCHX17*	*ZIFL1, AT3G58060, AT5G61520, AT5G18840*
transcription factor	11	*TCP15, TCP9, ARF11*	*bHLH093, NAC047, ATMYB75, SCL-3, TZF3, ATGT2L, ANAC079, BZS1*
enzyme regulatory factor	1	-	*AtCYS2*
growth factor activity	1	*PSK4*	-
nutrient reservoir activity	1	*PLP6*	-
function not assigned	39		
total	107		

Table 2. Genes involved in GA metabolism and signaling

TAIR ID	Canonic name	Other phytohormones that affect expression	Description
AT1G02400↑#	*GA2OX6*	IAA↑, ABA↑, JA↓	gibberellin 2-oxidase 6 (GA breakdown)
AT1G30040↑	*GA2OX2*		gibberellin 2-beta-dioxygenase 2 (GA breakdown)
AT1G15550↓	*GA3OX1*	JA↓, IAA↓	gibberellin 3-beta-dioxygenase 1 (GA biosynthesis)
AT4G25420↓	*GA20OX 1*	CK↑, IAA↑	gibberellin 20 oxidase 1 (GA biosynthesis)
AT5G51810↓	*GA20OX 2*	IAA↑	gibberellin 20 oxidase 2 (GA biosynthesis)
AT3G05120↓	*GID1A*	JA↑, IAA↓, ABA↓	putative gibberellin receptor GID1L1 (GA signal transduction)
AT3G63010↓	*GID1B*	JA↑, ABA↑	putative gibberellin receptor GID1L2 (GA signal transduction)
AT1G14920↑	*GAI*		DELLA protein GAI (repressor of GA responses)
AT4G24210↓	*SLY1*		F-box protein GID2 (GA signal transduction)
AT1G50420↓	*SCL3*		scarecrow-like protein 3 (positive regulator of GA responses, DELLA attenuator)

The arrows ↑↓ indicate upregulation or downregulation after GA_3 treatment in the case of genes while in the case of hormones - their effect on that particular gene.

Genes involved in cell wall remodeling and growth.

A number of genes from our list predominantly localized in the extracellular region were determined to be involved in remodeling of the cell wall which is crucial for assuring the plasticity needed for growth associated cell expansion (tabel 3). These genes have been found to be involved in acting on different polymer components of the cell wall.

For instance gene *AT3G07010* (upregulated) encodes a putative pectate lyase – an enzyme that catalyses the eliminative cleavage of de-esterified pectin, which is a major component (35% of the dry weight) of the primary cell wall of many higher plants (Carpita and Gibeaut, 1993). Originally, pectate lyases were associated mainly with plant pathogens whose secretions resulted in the maceration of plant tissues, but nowadays, they are assigned also with a more general role as facilitators of cell separation in a variety of physiological processes (Sun and van Nocker, 2010). Although the exact biological role for the members of this gene family is rarely known it is notable that the detailed study of a pectate lyase in *Zinnia elegans* has lead to the suggestion that these enzymes may assist in the removal and modification of the existing pectin matrix to allow the deposition of newly synthesized wall polymers for a specialized function (Domingo et al., 1998). Interestingly this study also proved upregulation of this gene by auxin which is also consistent with our data.
Inanition to this, PG3-a putative polygalacturonase ecoding gene was also upregulated by GA (tabel 3). Polygalacturonases degrade polygalacturonan which is a significant carbohydrate component of the pectin network.

The analysis has also found elevated expression levels for BGAL4. Studies show conistent involvement of β-galactosidase genes including BGAL4 in cells undergoing cell wall extension or remodeling in cotyledons, leaves and flower buds (Albornos et al., 2012; Gantulga et al., 2009). Interestingly, BGAL4 expression and secretion into the extracellular region is also induced by sugar starvation which, by the way, is associated with a decrease in the amount of monosaccharide in pectin and hemicellulose. This suggests its utilization by the cell as an energy/carbon source but also the potential of the enzyme to be used as a cell-wall remodeling factor (Lee et al., 2007). Indeed Down-regulation of tomato beta-galactosidase 4 results in decreased fruit softening (Smith et al., 2002).

Yet another gene affecting the pectic component of the cell wall is *AT5G38610* whose product is a plant invertase/pectin methylesterase inhibitor domain-containing protein and which was downregulated after GA treatment. Pectinesterases (pectin methyleseterases) catalyse the de-esterification of pectin into pectate and methanol and facilitate plant cell wall modification and subsequent breakdown.

Thus, it becomes evident that GA induces pectin modification by both activating a pectate lyase, a polygalacturonase, a β-galactosidase and by downregulating a repressor of pectinesterases.

While pectinesterases act on the cell wall they do so by primarily altering the localised pH of the cell wall. A low ph in the range of 3.0 to 5.0 is known to trigger cell-wall expansion by activating the catalytic activity of expansins (Durachko and Cosgrove, 2009; Wang et al., 2008). These are known to disrupt the cellulose-hemicellulose association transiently, allowing slippage or movement of cell wall polymers before the association reforms and the integrity of the cell wall network is reestablished.

Indeed, as expected, our list of genes contains an extracellularly located alpha expansin *EXP3* (tabel 3) which was found to be upregulated. *EXP3* was also found to be specifically upregulated along with other expansins in nematode-induced syncytia. These are multinuclear cells resulting either from endoreduplication or, in this case, from cell fusion, a process which requires cell-wall resorption as a prerequisite. In uninfected plants *EXPA3* has a shoot-specific expression (Wieczorek et al., 2006). Its upregulation by GA3, its downregulation by ABA and the mentioned expression pattern suggests also a potential role for this gene in remodeling the cell wall during gibberellin induced organ elongation.

Remodeling involves also other components of the cell wall matrix. For instance two members in our list of genes responsive to GA but also to other phytohormones are represented by xyloglucan endotransglucosylases/ hydrolases a class of enzymes with about 33 members in *Arabidopsis*. Leading cell wall models suggest that xyloglucan endo-transglycosylase (XET) activity is the primary catalyst (together with expansins) of controlled cell wall loosening through the transient cleavage and re-ligation of xyloglucan-cellulose cross-links (Kaewthai et al., 2013). The upregulated xyloglucan endotransglucosylase/hydrolase gene *XTH17* is known to be expressed evenly in all the cell types in the

elongating and differentiating regions of both the main and lateral roots, but not in the tip of these roots where cell division occurs (Vissenberg et al., 2005). Notably, the mutant in the *xth17* gene has low hemicellulose content (Zhu et al., 2014), probably as a result of impaired integration of new xyloglycan molecules into the polymer matrix of the cell wall. By contrast a second endotransglycosylase/hydrolase, *XTH32*, was downregulated. The transcripts of this gene might be involved in the rearrangement of cell walls of differentiating vessels elements (Irshad et al., 2008). However it seems to be dispensable for normal cell growth implying that morphological effects are subtle or may be compensated by other mechanisms (Kaewthai et al., 2013).

When analyzing the effect exerted by other phytohormones on the mentioned GA responsive genes, it becomes evident that ABA regulated antagonistically each of the genes in this list whose expression it affected (table 3), in line with its known effect in slowing down or halting growth (e.g shoot).

The generally counteracting role that the jasmonates demonstrate in relationship with GA is due to their involvement in stress response, both biotic and abiotic, which ultimately turns out into growth inhibition (e.g root growth) to allow the plant to channel its activity towards overcoming these particular stress conditions while delaying growth for a more favorable period. Indeed, recent research has revealed a molecular cascade involving COI1–JAZ–DELLA–PIF by which angiosperm plants prioritize JA-mediated defense over GA mediated growth (where COI1 and JAZ are upstream components of the jasmonate signaling pathway while DELLA and PIF – elements of the GA signaling pathway) (Yang et al., 2012).

At the same time a few genes responsive to GA, IAA and BR were regulated synergistically consistent with the role of this hormones in cell growth, organ elongation or stress counteraction.

Genes that determine turgor pressure

Remodeling of the cell wall components during cell growth must be accompanied by a high turgor pressure to allow the passive expansion of the polymer shell encapsulating the cell. This is implemented via accumulation of osmotically active substances, such as potassium ions (K+), sugars, and amino acids which are stored mainly in the vacuole (up to 90% of the cell volume). Indeed, a series of GA responsive genes from the list were found to be involved in cation transport across the cell or vacuole membrane, in response to water deprivation or salt stress (table 4).

K+ uptake and efflux represent a primary mechanism of osmotic regulation. The *A. thaliana* genome contains multigene families of K+ channels and transporters that have distinct or redundant functions (Maser et al., 2001), presumably due to high- and low-affinity K+ transport activity, tissue/cellular-specific gene expression, and protein subcellular localization (Lebaudy et al., 2007; Very and Sentenac, 2003). Despite identifying certain K +/ H+ exchangers (Andres et al., 2014), many transporters mediating the energetically uphill transfer of K+ across the cell membrane and into the vacuole remain to be identified.

The root specific *CHX17* gene which was upregulated according to our data encodes for a K +/ H+ exchanger and has a demonstrated role in K(+) acquisition and homeostasis (Cellier et al., 2004). *CHX17* expression is also induced by abscisic acid (ABA) (consistent with our expression data analysis) and external acidic pH (Cellier et al., 2004) – the same way as expansins. Furthermore the protein represents an antiporter that assures both intake of K+ cations and export of H+ cations further lowering the ph of the cell wall and representing a potential dual mechanism of integrating, on one hand, acidification of the cell wall necessary for its expansion and on the other hand, turgor pressure generation.

Among the downregulated genes was the ion transporter - *HLM1* known to be induced in response to pathogen infection and some pathogen-related signals and acting as a cGMP and cAMP gated channel permeable to both K(+) and Na(+) (Balague et al., 2003). Despite its role in defensive responses this gene could also serve routinely in regulation of osmotic potential and its downregulation by GA fits the idea that this phytohormone acts to retain and increase ion content in the cells. *HLM1* is also known to be downregulated by IAA and upregulated by jasmonate which again fits the classical relationship pattern of these phytohormones in growth regulation.
In addition to these, two cation efflux family proteins: AT3G58060 and AT1G79520 - both found to increase tolerance to divalent metal ions such as cadmium, zinc and cobalt were downregulated, the last one in an antagonistical manner to ABA (tabel 4).

Another H+-coupled K+ transporter, ZIFL1, was downregulated by GA but upregulated by JA and ABA. This protein localizes either to the tonoplast of root cells or to the plasma membrane of leaf stomatal guard cells

Table 3. Cell wall remodeling genes responsive to GA3

TAIR ID	Canonic name	Other phytohormones that affect expression	Description	Cellular location
AT2G36870↓ #	*XTH32*	CK↑, ABA↑, JA↓, IAA↓	xyloglucan:xyloglucosyl transferase	apoplast, cell wall, extracellular region
AT3G07010↑	*AT3G07010*	IAA↑, BR↑, JA↓, ABA↓	putative pectate lyase 8	extracellular
AT1G65310↑	*XTH17*	IAA↑, BR↑, JA↓	xyloglucan:xyloglucosyl transferase	apoplast, cell wall, extracellular region, plant-type cell wall
AT5G56870↑	*BGAL4*	JA↓, ABA↓	beta-galactosidase 4	extracellular region
AT2G37640↑	*EXP3*	ABA↓	expansin-A3	extracellular region
AT4G23920↓	*UGE2*	ABA↑	UDP-D-glucose/UDP-D-galactose 4-epimerase 2	cytosol, plasma membrane
AT1G23760↑	*PG3*		putative polygalacturonase	extracellular region
AT5G38610↓	*AT5G38610*		plant invertase/pectin methylesterase inhibitor domain-containing protein	extracellular region

Table 4. Genes involved in regulating the osmotic potential

TAIR ID	Canonic name	Other phytohormones that affect expression	Description	Cellular location
AT5G54250↓	HLM1	JA↑, IAA↓	cyclic nucleotide-gated ion channel 4	membrane, plasma membrane
AT1G79520↓	AT1G79520	ABA↑	Cation efflux family protein	integral to membrane, membrane, nucleus, plasma membrane
AT4G23700↑	CHX17	ABA↑	cation/H(+) antiporter 17	integral to membrane, late endosome, nucleus
AT3G58060↓	AT3G58060		putative metal tolerance protein C3	integral to membrane, membrane, nucleus
AT5G13750↓	ZIFL1	JA↑, ABA↑, CK↓	zinc induced facilitator-like 1 protein	plant-type vacuole membrane, plasma membrane

depending on the splicing variant however its main function is proposed to be involvement in polar auxin transport by modulating potassium and proton fluxes. The guard cell ZIFL1 isoform mediates drought tolerance by regulating stomatal closure (Remy et al., 2013).

Three more genes from the list, Xerico and XF1, and WNK (not shown in table 4) are known to be responsive to water deprivation (TAIR database).

In general ABA and jasmonate levels are elevated manyfold during drought stress which is suggestive of their role in promoting drought tolerance responses such as osmotic adjustment which implies retention of ions in the cell, however the mechanisms and consequently the regulatory actions for these processes may be different or only partially overlapping with the mechanisms involved in growth.

In spite of the data available about the mentioned genes that state their involvement in specific processes it is very probable that they have additional, yet unidentified roles one of which could be the coordinated action in the GA-induced growth associated regulation of osmotic potential.

Gibberellin and ethylene mediate endoreduplication via multiple pathways

Our analysis of microarray expression data for the GA3-treated *ga1-5 Arabidopsis* mutant revealed two TCP (CINCINNATA-like TEOSINTE BRANCHED1-CYCLOIDEA-PCF) family transcription factor genes, namely *TCP9* and *TCP15*, which were both upregulated by gibberellins (figures 2 and 3).

Also, our results showed that *TCP9* is upregulated by jasmonate and *TCP15* is downregulated by ethylene. The TCP family of plant-specific, non-canonical bHLH transcription factors (Cubas et al., 1999; Martin-Trillo and Cubas, 2010) has been specifically linked to the regulation of cell proliferation and expansion during development (Herve et al., 2009; Li et al., 2005). While TCP9 is involved in regulating senescence, the functionally related TCP14 and TCP15 factors are known to act redundantly in determining plant stature by promoting cell division in young internodes and were shown to modulate cell proliferation in the developing leaf blade and specific floral tissues (Kieffer et al., 2011). In addition, TCP15 is linked to the transition between two antagonistic states: mitosis and endoreduplication. Considering this but also the functional assignment of other GA responsive genes from our list, that was described above, we proposed that several major plant physiological processes that occur under GA cross talk with other phytohormones are related to cell cycle, cell growth/ elongation and, importantly, to the transition from mitotic cell cycle to endocycles.

Endoreduplication in plants plays an important role in developmental processes, in cell differentiation, in growth, metabolism, tissue morphogenesis and regeneration. For instance, plants use endoreduplication as a differentiation program oriented towards providing the embryo with necessary nutrients and proteins. Endosperm formation happens immediately after fertilization and is associated with the transition from mitotic cycles to endocycles (Grafi and Larkins, 1995; Leiva-Neto et al., 2004). In addition to this, suspensor cells, which use endoreplication to ensure poliploidy, function along with the endosperm to nourish the embryo. Endoreduplication happens also in tissues that rapidly acquire mass or have a high metabolic activity (Inze and De Veylder, 2006). The rapid growth of the hypocotyl during its emergence out of the soil before the onset of photosynthesis and absorption through the root is also assured by endoreduplication (Jakoby and Schnittger, 2004). Endoreduplication is widespread in tissues associated with absorption and storage of nutrients for instance in leaf and root trichomes where endoreduplication has the role of increasing the absorption surface of either light or water (Kondorosi et al., 2000).

The first evidence that endoreduplication is regulated by ethylene and gibberellin by modulating the activity of CDKs and other cell-cycle-related factors was first reported by (Gendreau et al., 1999). They showed that: (i) low levels of GA have a global positive effect on endoreduplication, whereas high levels restored normal cell growth and division; (ii) ethylene induces extra rounds of endoreduplication; (iii) the GA-insensitive mutant *gai* (lacking the DELLA region) showed reduced cell elongation but normal ploidy levels.

Earlier reports also showed that DELLA proteins contribute to growth inhibition and are able to control cell proliferation rates by regulating KRP2 (Kip-Related Protein2), SIM (SIAMESE), SIM-RELATED1 (SMR1), and SMR2 transcription (Achard et al., 2009). Finally, (Claeys et al., 2012), showed that mitotic exit of proliferating cells is fully DELLA-dependent and is controlled by APC/C (anaphase-promoting complex/cyclosome, a multisubunit ubiquitin E3 ligase that regulates the progression of cell cycles) which is in turn controlled by DEL1 and UVI4 (Claeys et al., 2012). In turn, the transcriptional control of the DEL1 gene is carried by the E2Fb and E2Fc transcription factors with antagonistic functions (Berckmans et al., 2011; Vlieghe et al., 2005). Ethylene, at the same, time determines the translocation of protein COP1 into the nucleus where it degrades E2Fb. In the absence of E2Fb, the E2Fc-DP complex blocks the transcription of DEL1. The decrease in DEL1 levels allows cells to enter the endoreduplication cycle (Berckmans et al., 2011).

Other results show that UVI4 also regulates the APC/C activity by binding to the CCS52A activator subunit. UVI4 is under the direct regulation of the E2F pathway and suppresses endocycle onset by inhibiting CYCA2;3 degradation (Heyman et al., 2011). It is known that the knockout of CYCA2;3 promotes endocycles and inactivation of RBR (RETINOBLASTOMA RELATED) induces extra-endoreduplication (Desvoyes et al., 2006; Imai et al., 2006; Li et al., 2012b). The TCP15 protein binds directly to the promoter regions of CYCA2;3 and RBR genes thus suppressing endoreduplication and activating cell proliferation.
To evaluate the role of GA and its cross talk with ethylene, we review existing knowledge of the process integrating it with our observations and proceed with describing three possible mechanisms that promote and control the mitosis to endoreduplication transition (figure 3).

Regulation via the anaphase-promoting complex/cyclosome (APC/C)

One of the major cross talk events between gibberellin and ethylene is represented by the interaction of DEL1/UVI4 (that are under DELLA negative control)

[Barbara et al., 2011] with the E2F transcription factor family whose abundance is under ethylene control (Parapunova et al., 2014). This interaction via the APC/C complex modulates the level of M-CDK (Heyman et al., 2011) and, thus, favors one of the two possible outputs – mitosis or endoreduplication. We can conclude that a high level of gibberellins leads to DELLA degradation and DEL1/UVI4 accumulation. This events allow DEL1 and UVI4 to interact with the CCS52A subunit of the APC/C complex and block degradation of M-CDK cyclins normally associated with mitosis progression (figure 3, A, D).

Upregulation of cell cycle inhibitors by GA signaling

DELLA proteins function as global repressors of plant growth by reducing cell proliferation and expansion rates and represent important hubs in phytohormone cross talk. (Achard et al., 2009) showed that DELLAs restrain cell proliferation by increasing the abundance of Kip-related protein 2 (KRP2) and SIAMESE (SIM) cell cycle inhibitors which have been shown to control endoreduplication in *Arabidopsis* trichomes (Churchman et al., 2006) and can interact with CDKB/cyclin complexes (Van Leene et al., 2010). In this way, developmental transition from mitotic cycles to endocycles is triggered by a decrease in mitotic (B-type) cyclin-dependent kinase (CDK) activity (Breuer et al., 2010; De Veylder et al., 2007). Thus, gibberellins via the DELLA proteins control cell proliferation by modulating the activity of CYC–CDK complexes (figure 3, B).

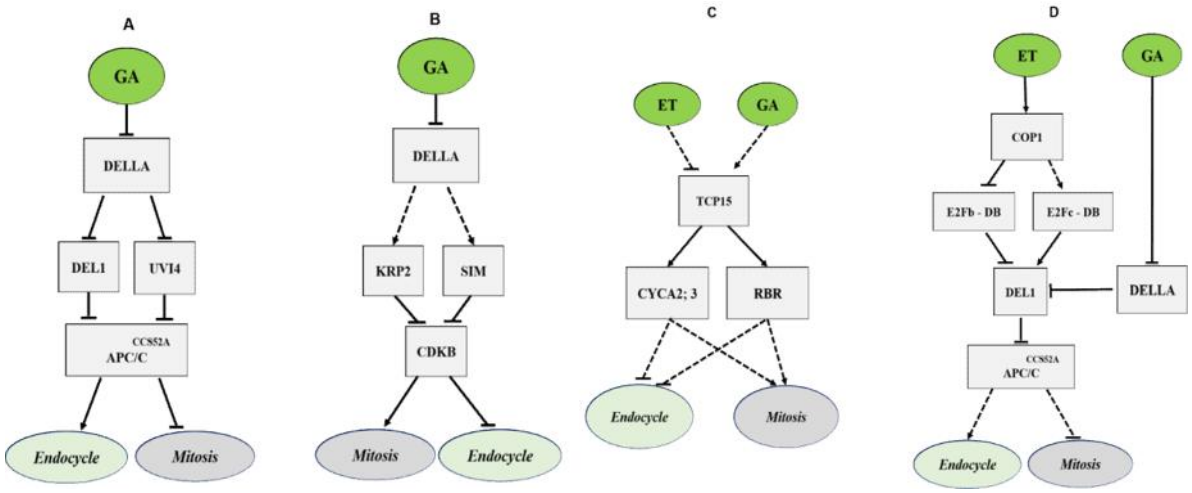


Figure 3. Role of gibberellin and ethylene in the mitosis to endoreduplication transition.
The control of this transition can be achieved via the anaphase-promoting complex/cyclosome (APC/C) (A, D); upregulation of cell cycle inhibitors by GA signalling (B) and by transcriptional control of CYCA2;3 and RBR by ethylene (C).

Transcriptional control of CYCA2;3 and RBR by ethylene

Finally, the TCP15 transcription factor was described to have a role in regulation of endoreduplication. According to (Li et al., 2012b) overexpression of TCP15 suppresses endoreduplication, while inactivation of this factor results in larger cells and increased levels of ploidy. The same authors used a chromatin immunoprecipitation (ChIP) assay to show that the TCP15 protein was bound at site-specific chromatin locations in CYCA2;3 and RBR promoters and modulated the expression of several cell-cycle genes (Desvoyes et al., 2006; Imai et al., 2006). This is another way to maintain endoreduplication.

Interestingly, the promoter region of *TCP15* contains a recognition site for the product of the ethylene-responsive gene *CEJ1* (COOPERATIVELY REGULATED BY ETHYLENE AND JASMONATE 1) (Parapunova et al., 2014). Taking into account that *CEJ1* is upregulated as a response to ethylene (Nakano et al., 2006) and our data according to which *TCP15* was downregulated by ethylene we suggest that *TCP15* expression is directly and negatively regulated by CEJ1 (figure 3, C). Recently published data also uncovered the interaction with and suggested modification of TCP15 (but also the closely related TCP14) by SPINDLY, an O-linked N-acetylglucosamine (O-GlcNAc) transferase acting as a DELLA independent repressor of GA

responses (Steiner et al., 2012). SPY was also shown to promote CK signaling (Jacobsen and Olszewski, 1993) and it seems to act so in concert with TCP14/15. Thus, whereas TCP14-overexpressing plants were hypersensitive to cytokinins, *spy* and *tcp14 tcp15* double mutant leaves and flowers were hyposensitive to the hormone which highlights the crucial role of SPY-dependent TCP14 and TCP15 activities in the response to cytokinins (Steiner et al., 2012). All the facts mentioned above demonstrate the involvement of TCP15 with multiple phytohormone signaling pathways both as a target and an effector.

Resuming the above mentioned data, our analysis highlights three mechanisms by which gibberellins may control the edoreduplication-mitosis transition mechanism: (i) in big quantities GAs indirectly activate the TCP15 transcription factor and thus favors mitosis by maintaining the function of the CYCA2;3 and RBR genes. This effect is antagonistic to ethylene which is a transcriptional repressor of TCP15 and, in the absence of the last, the expression of the factors necessary for promoting mitosis doesn't happen and thus the transition to endoreduplication occurs; (ii) in small quantities GAs favor endoreduplication by means of cytokinin degradation (M-CDK) which is normally contributing to the advancement of the mitotic cycle and thus favoring the transition from mitosis to endoreduplication. These possible mechanisms are in agreement and expand on the results of Gendreau et al., 1999; (iii) the third way of endoreduplication promotion by gibberellins is activated by the DELLA family proteins, which indirectly activate cyclin inhibitors SIM and KRP2. Diminishing of the cyclin dependent kinase mitotic activity (type B) ensures the transition from mitotic cycles to endocycles.

Conclusion

In this study we have identified a list of GA_3 responsive genes in 7-day *Arabidopsis* seedlings of the *ga1*-5 weak gibberellin biosynthesis mutant by using bioinformatics instruments to analyze publicly available microarray expression data. This allowed us to extend and integrate previously published data regarding the role of gibberellins in plant growth and development.

The obtained list of genes contained multiple actors involved in GA metabolism and signaling regulated in a manner intended to diminish excessive signaling on multiple levels. These genes were also differentially regulated by other phytohormones mainly by jasmonate, auxin and abscisic acid in a complex, individual manner. The analysis also revealed a large group of genes involved in remodeling various macromolecular components of the cell wall, some of them only loosely associated with growth processes in previous studies but all of them sharing a common function of acting on the cell wall matrix. Additionally, a group of actors was revealed with potential role in modulating the osmotic potential (turgor pressure) of the cells. The regulation of gene expression involved in cell wall remodeling has revealed strong antagonism of gibberellins with abscisic acid and jasmonate and good synergism with auxin.

The significant number of GA responsive genes whose expression levels were also affected by other phytohormones (70% of all the differentially expressed genes) indicates that gibberellin signaling largely overlaps with other phytohormone response pathways and has rather a role of signal modulator and integrator than that of a final an independent effector.

Finally, we confirm with our results the role of GA in endoreduplication, and integrate current knowledge to conclude that gibberellin acts via an elegant cross talk with ethylene either synergistically or antagonistically to control endoreduplication by means of multiple mechanisms. The existence of multiple pathways for controlling the mitosis to endoreduplication transition process may be explained by the fact that this process is tissue-specific and may depend on the ontogenetic stage of the plant organism or on environmental conditions. Also, some of the described pathways may participate in endoreduplication induction while others – in the maintenance or regulation of the extent of the process.

Acknowledgements

We would like to thank DUCA Maria, academician, professor and dean at the University of the Academy of Sciences of Moldova for assistance and criticism during the editing process of this paper.

References

1. Achard, P., Gong, F., Cheminant, S., Alioua, M., Hedden, P., and Genschik, P. (2008). The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. Plant Cell *20*, 2117-2129.
2. Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T., and Genschik, P. (2009). Gibberellin signaling

controls cell proliferation rate in Arabidopsis. Curr Biol *19*, 1188-1193.

3. Achard, P., Vriezen, W.H., Van Der Straeten, D., and Harberd, N.P. (2003). Ethylene regulates arabidopsis development via the modulation of DELLA protein growth repressor function. Plant Cell *15*, 2816-2825.
4. Alabadi, D., Gallego-Bartolome, J., Orlando, L., Garcia-Carcel, L., Rubio, V., Martinez, C., Frigerio, M., Iglesias-Pedraz, J.M., Espinosa, A., Deng, X.W., *et al.* (2008). Gibberellins modulate light signaling pathways to prevent Arabidopsis seedling de-etiolation in darkness. Plant J *53*, 324-335.
5. Albornos, L., Martin, I., Perez, P., Marcos, R., Dopico, B., and Labrador, E. (2012). Promoter activities of genes encoding beta-galactosidases from Arabidopsis a1 subfamily. Plant Physiol Biochem *60*, 223-232.
6. Andres, Z., Perez-Hormaeche, J., Leidi, E.O., Schlucking, K., Steinhorst, L., McLachlan, D.H., Schumacher, K., Hetherington, A.M., Kudla, J., Cubero, B., *et al.* (2014). Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake. Proc Natl Acad Sci U S A *111*, E1806-1814.
7. Arnaud, N., Girin, T., Sorefan, K., Fuentes, S., Wood, T.A., Lawrenson, T., Sablowski, R., and Ostergaard, L. (2010). Gibberellins control fruit patterning in Arabidopsis thaliana. Genes Dev *24*, 2127-2132.
8. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., *et al.* (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet *25*, 25-29.
9. Bai, M.Y., Shang, J.X., Oh, E., Fan, M., Bai, Y., Zentella, R., Sun, T.P., and Wang, Z.Y. (2012). Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. Nat Cell Biol *14*, 810-817.
10. Balague, C., Lin, B., Alcon, C., Flottes, G., Malmstrom, S., Kohler, C., Neuhaus, G., Pelletier, G., Gaymard, F., and Roby, D. (2003). HLM1, an essential signaling component in the hypersensitive response, is a member of the cyclic nucleotide-gated channel ion channel family. Plant Cell *15*, 365-379.
11. Berckmans, B., Lammens, T., Van Den Daele, H., Magyar, Z., Bogre, L., and De Veylder, L. (2011). Light-dependent regulation of DEL1 is determined by the antagonistic action of E2Fb and E2Fc. Plant Physiol *157*, 1440-1451.
12. Breuer, C., Ishida, T., and Sugimoto, K. (2010). Developmental control of endocycles and cell growth in plants. Curr Opin Plant Biol *13*, 654-660.
13. Carlson, M. (2013). ath1121501.db: Affymetrix Arabidopsis ATH1 Genome Array annotation data (chip ath1121501). R package version 2.14.0.
14. Carpita, N.C., and Gibeaut, D.M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J *3*, 1-30.
15. Cellier, F., Conejero, G., Ricaud, L., Luu, D.T., Lepetit, M., Gosti, F., and Casse, F. (2004). Characterization of AtCHX17, a member of the cation/H+ exchangers, CHX family, from Arabidopsis thaliana suggests a role in K+ homeostasis. Plant J *39*, 834-846.
16. Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D.E., Cao, D., Luo, D., Harberd, N.P., and Peng, J. (2004). Gibberellin regulates Arabidopsis floral development via suppression of DELLA protein function. Development *131*, 1055-1064.
17. Churchman, M.L., Brown, M.L., Kato, N., Kirik, V., Hulskamp, M., Inze, D., De Veylder, L., Walker, J.D., Zheng, Z., Oppenheimer, D.G., *et al.* (2006). SIAMESE, a plant-specific cell cycle regulator, controls endoreplication onset in Arabidopsis thaliana. Plant Cell *18*, 3145-3157.
18. Claeys, H., Skirycz, A., Maleux, K., and Inze, D. (2012). DELLA signaling mediates stress-induced cell differentiation in Arabidopsis leaves through modulation of anaphase-promoting complex/cyclosome activity. Plant Physiol *159*, 739-747.
19. Cubas, P., Lauter, N., Doebley, J., and Coen, E. (1999). The TCP domain: a motif found in proteins regulating plant growth and development. Plant J *18*, 215-222.
20. de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blazquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature *451*, 480-484.
21. De Veylder, L., Beeckman, T., and Inze, D. (2007). The ins and outs of the plant cell cycle. Nat Rev Mol Cell Biol *8*, 655-665.
22. Desvoyes, B., Ramirez-Parra, E., Xie, Q., Chua, N.H., and Gutierrez, C. (2006). Cell type-

specific role of the retinoblastoma/E2F pathway during Arabidopsis leaf development. Plant Physiol *140*, 67-80.

23. Dill, A., and Sun, T. (2001). Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. Genetics *159*, 777-785.
24. Dill, A., Thomas, S.G., Hu, J., Steber, C.M., and Sun, T.P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell *16*, 1392-1405.
25. Domingo, C., Roberts, K., Stacey, N.J., Connerton, I., Ruiz-Teran, F., and McCann, M.C. (1998). A pectate lyase from Zinnia elegans is auxin inducible. Plant J *13*, 17-28.
26. Durachko, D.M., and Cosgrove, D.J. (2009). Measuring plant cell wall extension (creep) induced by acidic pH and by alpha-expansin. J Vis Exp, 1263.
27. Edgar, R., Domrachev, M., and Lash, A.E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res *30*, 207-210.
28. Farquharson, K.L. (2010). Gibberellin-auxin crosstalk modulates lateral root formation. Plant Cell *22*, 540.
29. Feng, S., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J., Wang, F., Chen, L., Yu, L., Iglesias-Pedraz, J.M., Kircher, S., *et al.* (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature *451*, 475-479.
30. Frigerio, M., Alabadi, D., Perez-Gomez, J., Garcia-Carcel, L., Phillips, A.L., Hedden, P., and Blazquez, M.A. (2006). Transcriptional regulation of gibberellin metabolism genes by auxin signaling in Arabidopsis. Plant Physiol *142*, 553-563.
31. Galinha, C., Bilsborough, G., and Tsiantis, M. (2009). Hormonal input in plant meristems: A balancing act. Semin Cell Dev Biol *20*, 1149-1156.
32. Gallego-Bartolome, J., Alabadi, D., and Blazquez, M.A. (2011). DELLA-induced early transcriptional changes during etiolated development in Arabidopsis thaliana. PLoS One *6*, e23918.
33. Gallego-Bartolome, J., Minguet, E.G., Grau-Enguix, F., Abbas, M., Locascio, A., Thomas, S.G., Alabadi, D., and Blazquez, M.A. (2012). Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in Arabidopsis. Proc Natl Acad Sci U S A *109*, 13446-13451.
34. Gantulga, D., Ahn, Y.O., Zhou, C., Battogtokh, D., Bevan, D.R., Winkel, B.S., and Esen, A. (2009). Comparative characterization of the Arabidopsis subfamily a1 beta-galactosidases. Phytochemistry *70*, 1999-2009.
35. Gautier, L., Cope, L., Bolstad, B.M., and Irizarry, R.A. (2004). affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics *20*, 307-315.
36. Gendreau, E., Orbovic, V., Hofte, H., and Traas, J. (1999). Gibberellin and ethylene control endoreduplication levels in the Arabidopsis thaliana hypocotyl. Planta *209*, 513-516.
37. Goda, H., Sasaki, E., Akiyama, K., Maruyama-Nakashita, A., Nakabayashi, K., Li, W., Ogawa, M., Yamauchi, Y., Preston, J., Aoki, K., *et al.* (2008). The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. Plant J *55*, 526-542.
38. Grafi, G., and Larkins, B.A. (1995). Endoreduplication in maize endosperm: involvement of m phase--promoting factor inhibition and induction of s phase--related kinases. Science *269*, 1262-1264.
39. Greenboim-Wainberg, Y., Maymon, I., Borochov, R., Alvarez, J., Olszewski, N., Ori, N., Eshed, Y., and Weiss, D. (2005). Cross talk between gibberellin and cytokinin: the Arabidopsis GA response inhibitor SPINDLY plays a positive role in cytokinin signaling. Plant Cell *17*, 92-102.
40. Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., *et al.* (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell *18*, 3399-3414.
41. Hedden, P., and Phillips, A.L. (2000). Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci *5*, 523-530.
42. Heinrich, M., Hettenhausen, C., Lange, T., Wunsche, H., Fang, J., Baldwin, I.T., and Wu, J. (2013). High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of Nicotiana attenuata stems. Plant J *73*, 591-606.
43. Herve, C., Dabos, P., Bardet, C., Jauneau, A., Auriac, M.C., Ramboer, A., Lacout, F., and Tremousaygue, D. (2009). In vivo interference with AtTCP20 function induces severe plant

growth alterations and deregulates the expression of many genes important for development. Plant Physiol *149*, 1462-1477.

44. Heyman, J., Van den Daele, H., De Wit, K., Boudolf, V., Berckmans, B., Verkest, A., Alvim Kamei, C.L., De Jaeger, G., Koncz, C., and De Veylder, L. (2011). Arabidopsis ULTRAVIOLET-B-INSENSITIVE4 maintains cell division activity by temporal inhibition of the anaphase-promoting complex/cyclosome. Plant Cell *23*, 4394-4410.
45. Hou, X., Lee, L.Y., Xia, K., Yan, Y., and Yu, H. (2010). DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell *19*, 884-894.
46. Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009a). Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res *37*, 1-13.
47. Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc *4*, 44-57.
48. Ikeda, A., Ueguchi-Tanaka, M., Sonoda, Y., Kitano, H., Koshioka, M., Futsuhara, Y., Matsuoka, M., and Yamaguchi, J. (2001). slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the SLR1 gene, an ortholog of the height-regulating gene GAI/RGA/RHT/D8. Plant Cell *13*, 999-1010.
49. Imai, K.K., Ohashi, Y., Tsuge, T., Yoshizumi, T., Matsui, M., Oka, A., and Aoyama, T. (2006). The A-type cyclin CYCA2;3 is a key regulator of ploidy levels in Arabidopsis endoreduplication. Plant Cell *18*, 382-396.
50. Inze, D., and De Veylder, L. (2006). Cell cycle regulation in plant development. Annu Rev Genet *40*, 77-105.
51. Irizarry, R.A., Hobbs, B., Collin, F., Beazer-Barclay, Y.D., Antonellis, K.J., Scherf, U., and Speed, T.P. (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics *4*, 249-264.
52. Irshad, M., Canut, H., Borderies, G., Pont-Lezica, R., and Jamet, E. (2008). A new picture of cell wall protein dynamics in elongating cells of Arabidopsis thaliana: confirmed actors and newcomers. BMC Plant Biol *8*, 94.
53. Jacobsen, S.E., and Olszewski, N.E. (1993). Mutations at the SPINDLY locus of Arabidopsis alter gibberellin signal transduction. Plant Cell *5*, 887-896.
54. Jakoby, M., and Schnittger, A. (2004). Cell cycle and differentiation. Curr Opin Plant Biol *7*, 661-669.
55. Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P., and Tsiantis, M. (2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr Biol *15*, 1560-1565.
56. Kaewthai, N., Gendre, D., Eklof, J.M., Ibatullin, F.M., Ezcurra, I., Bhalerao, R.P., and Brumer, H. (2013). Group III-A XTH genes of Arabidopsis encode predominant xyloglucan endohydrolases that are dispensable for normal growth. Plant Physiol *161*, 440-454.
57. Kieffer, M., Master, V., Waites, R., and Davies, B. (2011). TCP14 and TCP15 affect internode length and leaf shape in Arabidopsis. Plant J *68*, 147-158.
58. King, K.E., Moritz, T., and Harberd, N.P. (2001). Gibberellins are not required for normal stem growth in Arabidopsis thaliana in the absence of GAI and RGA. Genetics *159*, 767-776.
59. Kohli, A., Sreenivasulu, N., Lakshmanan, P., and Kumar, P.P. (2013). The phytohormone crosstalk paradigm takes center stage in understanding how plants respond to abiotic stresses. Plant Cell Rep *32*, 945-957.
60. Kondorosi, E., Roudier, F., and Gendreau, E. (2000). Plant cell-size control: growing by ploidy? Curr Opin Plant Biol *3*, 488-492.
61. Koornneef, M., and van der Veen, J.H. (1980). Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) heynh. Theor Appl Genet *58*, 257-263.
62. Lamesch, P., Berardini, T.Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Muller, R., Dreher, K., Alexander, D.L., Garcia-Hernandez, M., *et al.* (2012). The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. Nucleic Acids Res *40*, D1202-1210.
63. Lebaudy, A., Very, A.A., and Sentenac, H. (2007). K+ channel activity in plants: genes, regulations and functions. FEBS Lett *581*, 2357-2366.
64. Lechner, E., Achard, P., Vansiri, A., Potuschak, T., and Genschik, P. (2006). F-box proteins everywhere. Curr Opin Plant Biol *9*, 631-638.
65. Lee, E.J., Matsumura, Y., Soga, K., Hoson, T., and Koizumi, N. (2007). Glycosyl hydrolases of cell wall are induced by sugar starvation in Arabidopsis. Plant Cell Physiol *48*, 405-413.

66. Lee, S., Cheng, H., King, K.E., Wang, W., He, Y., Hussain, A., Lo, J., Harberd, N.P., and Peng, J. (2002). Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes Dev *16*, 646-658.
67. Leiva-Neto, J.T., Grafi, G., Sabelli, P.A., Dante, R.A., Woo, Y.M., Maddock, S., Gordon-Kamm, W.J., and Larkins, B.A. (2004). A dominant negative mutant of cyclin-dependent kinase A reduces endoreduplication but not cell size or gene expression in maize endosperm. Plant Cell *16*, 1854-1869.
68. Li, C., Potuschak, T., Colon-Carmona, A., Gutierrez, R.A., and Doerner, P. (2005). Arabidopsis TCP20 links regulation of growth and cell division control pathways. Proc Natl Acad Sci U S A *102*, 12978-12983.
69. Li, Q.F., Wang, C., Jiang, L., Li, S., Sun, S.S., and He, J.X. (2012a). An interaction between BZR1 and DELLAs mediates direct signaling crosstalk between brassinosteroids and gibberellins in Arabidopsis. Sci Signal *5*, ra72.
70. Li, Z.Y., Li, B., and Dong, A.W. (2012b). The Arabidopsis transcription factor AtTCP15 regulates endoreduplication by modulating expression of key cell-cycle genes. Mol Plant *5*, 270-280.
71. Martin-Trillo, M., and Cubas, P. (2010). TCP genes: a family snapshot ten years later. Trends Plant Sci *15*, 31-39.
72. Maser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K., Sze, H., Talke, I.N., Amtmann, A., Maathuis, F.J., Sanders, D., *et al.* (2001). Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol *126*, 1646-1667.
73. Nakajima, M., Shimada, A., Takashi, Y., Kim, Y.C., Park, S.H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M., *et al.* (2006). Identification and characterization of Arabidopsis gibberellin receptors. Plant J *46*, 880-889.
74. Nakano, T., Suzuki, K., Ohtsuki, N., Tsujimoto, Y., Fujimura, T., and Shinshi, H. (2006). Identification of genes of the plant-specific transcription-factor families cooperatively regulated by ethylene and jasmonate in Arabidopsis thaliana. J Plant Res *119*, 407-413.
75. Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y., and Yamaguchi, S. (2003). Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell *15*, 1591-1604.
76. Parapunova, V., Busscher, M., Busscher-Lange, J., Lammers, M., Karlova, R., Bovy, A.G., Angenent, G.C., and de Maagd, R.A. (2014). Identification, cloning and characterization of the tomato TCP transcription factor family. BMC Plant Biol *14*, 157.
77. Peng, J. (2009). Gibberellin and jasmonate crosstalk during stamen development. J Integr Plant Biol *51*, 1064-1070.
78. Peng, J., Carol, P., Richards, D.E., King, K.E., Cowling, R.J., Murphy, G.P., and Harberd, N.P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev *11*, 3194-3205.
79. Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., Beales, J., Fish, L.J., Worland, A.J., Pelica, F., *et al.* (1999). 'Green revolution' genes encode mutant gibberellin response modulators. Nature *400*, 256-261.
80. Qi, T., Huang, H., Wu, D., Yan, J., Qi, Y., Song, S., and Xie, D. (2014). Arabidopsis DELLA and JAZ proteins bind the WD-repeat/bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. Plant Cell *26*, 1118-1133.
81. Remy, E., Cabrito, T.R., Baster, P., Batista, R.A., Teixeira, M.C., Friml, J., Sa-Correia, I., and Duque, P. (2013). A major facilitator superfamily transporter plays a dual role in polar auxin transport and drought stress tolerance in Arabidopsis. Plant Cell *25*, 901-926.
82. Richter, R., Behringer, C., Zourelidou, M., and Schwechheimer, C. (2013). Convergence of auxin and gibberellin signaling on the regulation of the GATA transcription factors GNC and GNL in Arabidopsis thaliana. Proc Natl Acad Sci U S A *110*, 13192-13197.
83. Rieu, I., Eriksson, S., Powers, S.J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S.G., Hedden, P., *et al.* (2008a). Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. Plant Cell *20*, 2420-2436.
84. Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S.J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S.G., *et al.* (2008b). The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and

development throughout the Arabidopsis life cycle. Plant J *53*, 488-504.

85. Rogers, J.C., and Rogers, S.W. (1992). Definition and functional implications of gibberellin and abscisic acid cis-acting hormone response complexes. Plant Cell *4*, 1443-1451.
86. Sasaki, A., Itoh, H., Gomi, K., Ueguchi-Tanaka, M., Ishiyama, K., Kobayashi, M., Jeong, D.H., An, G., Kitano, H., Ashikari, M., *et al.* (2003). Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science *299*, 1896-1898.
87. Seo, M., Nambara, E., Choi, G., and Yamaguchi, S. (2009). Interaction of light and hormone signals in germinating seeds. Plant Mol Biol *69*, 463-472.
88. Shan, X., Yan, J., and Xie, D. (2012). Comparison of phytohormone signaling mechanisms. Curr Opin Plant Biol *15*, 84-91.
89. Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res *13*, 2498-2504.
90. Silverstone, A.L., Jung, H.S., Dill, A., Kawaide, H., Kamiya, Y., and Sun, T.P. (2001). Repressing a repressor: gibberellin-induced rapid reduction of the RGA protein in Arabidopsis. Plant Cell *13*, 1555-1566.
91. Singh, R., and Jwa, N.S. (2013). Understanding the responses of rice to environmental stress using proteomics. J Proteome Res *12*, 4652-4669.
92. Smith, D.L., Abbott, J.A., and Gross, K.C. (2002). Down-regulation of tomato beta-galactosidase 4 results in decreased fruit softening. Plant Physiol *129*, 1755-1762.
93. Smyth GK (2005). Limma: linear models for microarray data. In Gentleman R, Carey V, Dudoit S, Irizarry R and Huber W (eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor, pp. 397–420. Springer, New York.
94. Steiner, E., Efroni, I., Gopalraj, M., Saathoff, K., Tseng, T.S., Kieffer, M., Eshed, Y., Olszewski, N., and Weiss, D. (2012). The Arabidopsis O-linked N-acetylglucosamine transferase SPINDLY interacts with class I TCPs to facilitate cytokinin responses in leaves and flowers. Plant Cell *24*, 96-108.
95. Sun, L., and van Nocker, S. (2010). Analysis of promoter activity of members of the PECTATE LYASE-LIKE (PLL) gene family in cell separation in Arabidopsis. BMC Plant Biol *10*, 152.
96. Tyler, L., Thomas, S.G., Hu, J., Dill, A., Alonso, J.M., Ecker, J.R., and Sun, T.P. (2004). Della proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol *135*, 1008-1019.
97. Valdovinos, J.G., Ernest, L.C., and Henry, E.W. (1967). Effect of ethylene and gibberellic Acid on auxin synthesis in plant tissues. Plant Physiol *42*, 1803-1806.
98. Van Huizen, R., Ozga, J.A., and Reinecke, D.M. (1996). Influence of Auxin and Gibberellin on in Vivo Protein Synthesis during Early Pea Fruit Growth. Plant Physiol *112*, 53-59.
99. Van Leene, J., Hollunder, J., Eeckhout, D., Persiau, G., Van De Slijke, E., Stals, H., Van Isterdael, G., Verkest, A., Neirynck, S., Buffel, Y., *et al.* (2010). Targeted interactomics reveals a complex core cell cycle machinery in Arabidopsis thaliana. Mol Syst Biol *6*, 397.
100. Very, A.A., and Sentenac, H. (2003). Molecular mechanisms and regulation of K+ transport in higher plants. Annu Rev Plant Biol *54*, 575-603.
101. Vissenberg, K., Oyama, M., Osato, Y., Yokoyama, R., Verbelen, J.P., and Nishitani, K. (2005). Differential expression of AtXTH17, AtXTH18, AtXTH19 and AtXTH20 genes in Arabidopsis roots. Physiological roles in specification in cell wall construction. Plant Cell Physiol *46*, 192-200.
102. Vlieghe, K., Boudolf, V., Beemster, G.T., Maes, S., Magyar, Z., Atanassova, A., de Almeida Engler, J., De Groodt, R., Inze, D., and De Veylder, L. (2005). The DP-E2F-like gene DEL1 controls the endocycle in Arabidopsis thaliana. Curr Biol *15*, 59-63.
103. Voegele, A., Linkies, A., Muller, K., and Leubner-Metzger, G. (2011). Members of the gibberellin receptor gene family GID1 (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during Lepidium sativum and Arabidopsis thaliana seed germination. J Exp Bot *62*, 5131-5147.
104. Wang, C.X., Wang, L., McQueen-Mason, S.J., Pritchard, J., and Thomas, C.R. (2008). pH and expansin action on single suspension-cultured tomato (Lycopersicon esculentum) cells. J Plant Res *121*, 527-534.
105. Weiss, D., and Ori, N. (2007). Mechanisms of cross talk between gibberellin and other hormones. Plant Physiol *144*, 1240-1246.

106. Wen, C.K., and Chang, C. (2002). Arabidopsis RGL1 encodes a negative regulator of gibberellin responses. Plant Cell *14*, 87-100.
107. Wieczorek, K., Golecki, B., Gerdes, L., Heinen, P., Szakasits, D., Durachko, D.M., Cosgrove, D.J., Kreil, D.P., Puzio, P.S., Bohlmann, H., *et al.* (2006). Expansins are involved in the formation of nematode-induced syncytia in roots of Arabidopsis thaliana. Plant J *48*, 98-112.
108. Wild, M., Daviere, J.M., Cheminant, S., Regnault, T., Baumberger, N., Heintz, D., Baltz, R., Genschik, P., and Achard, P. (2012). The Arabidopsis DELLA RGA-LIKE3 is a direct target of MYC2 and modulates jasmonate signaling responses. Plant Cell *24*, 3307-3319.
109. Willige, B.C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E.M., Maier, A., and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. Plant Cell *19*, 1209-1220.
110. Wilson, C.L., and Miller, C.J. (2005). Simpleaffy: a BioConductor package for Affymetrix Quality Control and data analysis. Bioinformatics *21*, 3683-3685.
111. Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annu Rev Plant Biol *59*, 225-251.
112. Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y., and Yamaguchi, S. (2004). Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of Arabidopsis thaliana seeds. Plant Cell *16*, 367-378.
113. Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L.J., Li, Q., Xiao, L.T., Sun, T.P., Li, J., Deng, X.W., *et al.* (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci U S A *109*, E1192-1200.
114. Yu, H., Ito, T., Zhao, Y., Peng, J., Kumar, P., and Meyerowitz, E.M. (2004). Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci U S A *101*, 7827-7832.
115. Yu, S., Galvao, V.C., Zhang, Y.C., Horrer, D., Zhang, T.Q., Hao, Y.H., Feng, Y.Q., Wang, S., Schmid, M., and Wang, J.W. (2012). Gibberellin regulates the Arabidopsis floral transition through miR156-targeted SQUAMOSA promoter binding-like transcription factors. Plant Cell *24*, 3320-3332.
116. Zentella, R., Zhang, Z.L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M., Jikumaru, Y., Nambara, E., Kamiya, Y., *et al.* (2007). Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. Plant Cell *19*, 3037-3057.
117. Zhang, Z.L., Ogawa, M., Fleet, C.M., Zentella, R., Hu, J., Heo, J.O., Lim, J., Kamiya, Y., Yamaguchi, S., and Sun, T.P. (2011). Scarecrow-like 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis. Proc Natl Acad Sci U S A *108*, 2160-2165.
118. Zhu, X.F., Wan, J.X., Sun, Y., Shi, Y.Z., Braam, J., Li, G.X., and Zheng, S.J. (2014). Xyloglucan Endotransglucosylase-Hydrolase17 Interacts with Xyloglucan Endotransglucosylase-Hydrolase31 to Confer Xyloglucan Endotransglucosylase Action and Affect Aluminum Sensitivity in Arabidopsis. Plant Physiol *165*, 1566-1574.