Running head: A2-type CYCLIN control of hyponastic growth

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Ethylene-mediated regulation of A2-type CYCLINs modulates hyponastic growth in *Arabidopsis thaliana*

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One–Sentence Summary:

Ethylene-mediated A2-type CYCLIN regulation of cell proliferation confines the amplitude of *Arabidopsis thaliana* hyponastic leaf movement.
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Abstract

Upward leaf movement (hyponastic growth) is frequently observed in response to changing environmental conditions and can be induced by the phytohormone ethylene. Hyponasty results from differential growth; i.e., enhanced cell elongation at the proximal abaxial side of the petiole relative to the adaxial side. Here we characterise Enhanced Hyponasty-D (EHY-D), an activation-tagged Arabidopsis thaliana line with exaggerated hyponasty. This phenotype is associated with overexpression of the mitotic cyclin CYCLINA2;1 (CYCA2;1), which hints at a role for cell divisions in regulating hyponasty. Indeed, mathematical analysis suggested that the observed changes in abaxial cell elongation rates during ethylene treatment should result in a larger hyponastic amplitude than observed, unless a decrease in cell proliferation rate at the proximal abaxial side of the petiole relative to the adaxial side was implemented. Our model predicts that when this differential proliferation mechanism is disrupted by either ectopic overexpression or mutation of CYCA2;1, the hyponastic growth response becomes exaggerated. This is in accordance with experimental observations on CYCA2;1 overexpression lines and cyca2;1 knock-outs. We therefore propose a bipartite mechanism controlling leaf movement; Ethylene induces longitudinal cell expansion in the abaxial petiole epidermis to induce hyponasty and simultaneously affects its amplitude by controlling cell proliferation through CYCA2;1. Further corroborating the model, we found that ethylene treatment results in transcriptional down regulation of A2-type CYCLINs and propose that this, and possibly other regulatory mechanisms affecting CYCA2;1, may contribute to this attenuation of hyponastic growth.
Introduction

Plants have acquired mechanisms to adjust growth and secure reproduction under unfavourable environmental conditions. Among the strategies to avoid adverse conditions is upward leaf movement, called hyponastic growth. This leaf reorientation is driven by unequal growth rates between ad- and abaxial sides of the petiole (Cox et al., 2004; Polko et al., 2012a). Arabidopsis thaliana exhibits hyponasty upon several environmental signals, e.g. submergence, waterlogging, proximity of neighbouring vegetation, low red:far-red light ratio’s, reduced blue light fluence rates, low light intensities and high temperatures (Keller et al., 2011; Keuskamp et al., 2010; De Wit et al., 2012; Moreno et al., 2009; Koini et al., 2009; Millenaar et al., 2005, 2009; Mullen et al., 2006; Van Zanten et al., 2009; Vasseur et al., 2011; Rauf et al., 2013, Dornbusch et al., 2014). Hyponasty alleviates the impact of environmental stresses (Van Zanten et al., 2010a). During submergence it allows reestablishment of gas exchange with the atmosphere (e.g. Cox et al., 2003), at high plant densities it positions the leaves in better lit layers of the canopy to improve light interception (e.g. de Wit et al., 2012), and at high temperatures it improves the cooling capacity of the leaves (Crawford et al., 2012; Bridge et al., 2013). The cellular basis of hyponastic growth in Rumex palustris (Cox et al., 2004) and Arabidopsis (Polko et al., 2012a, Rauf et al., 2013) has been characterized. Ethylene causes reorientation of cortical microtubules (CMTs) in the petiole, which leads to longitudinal cell expansion in a ~2 mm-long epidermal cell zone at the proximal part of the abaxial side of the organ (Polko et al., 2012a).

The interactions between several hormones, e.g. ethylene, abscisic acid, gibberellins and auxin, in controlling hyponasty under various conditions have been studied (Mullen et al., 2006; Benschop et al., 2007; Millenaar et al., 2009; Van Zanten et al., 2009, 2010a; Pena-Castro et al., 2011). The volatile phytohormone ethylene is a key component in the complex regulatory network of hyponastic growth. Ethylene is the trigger and a positive regulator of hyponastic growth in submerged and waterlogged Arabidopsis (Millenaar et al., 2005, 2009; Van Zanten et al., 2010a, Rauf et al., 2013) and a negative regulator of high temperature-induced hyponasty (Van Zanten et al., 2009), but is not involved in low light-induced hyponastic growth in this species (Millenaar et al., 2009). Abscisic acid (ABA) antagonizes ethylene-induced hyponasty (Benschop et al. 2007) and is a positive regulator of high temperature-induced hyponastic growth (Van Zanten et al. 2009). The growth-promoting gibberellins positively regulate hyponastic response to all these three environmental signals (Pena-Castro et al., 2011), while auxins promote low light and high temperature-induced hyponastic growth (Millenaar et al. 2005; Koini et al. 2009; Van Zanten et al. 2009), as well as low red:far-red- and low blue light-induced hyponasty (Moreno et al., 2009; Keller et al., 2011). Finally brassinosteroids also positively regulate ethylene-induced hyponasty (Polko et al., 2013).

Despite the extensive knowledge on hormonal regulation of hyponasty, little is known about the molecular genetic mechanisms that drive this response. One notable exception is the study of Rauf et al. (2013), who showed that hyponastic growth in Arabidopsis in response to root waterlogging is controlled by the NAC transcription factor SPEEDY HYPONASTIC GROWTH (SHYG) that directly affects expression of the ethylene biosynthesis gene ACC OXIDASE5 (ACO5).
Here, we followed a forward genetic approach to identify novel components that control hyponastic growth in Arabidopsis. From a population of activation-tagged plants (Weigel et al., 2000) we isolated *Enhanced Hyponasty-D* (*EHY-D*), which showed exaggerated hyponasty under exogenous ethylene application, low light intensities and high temperature. We found that ectopic expression of the core cell cycle regulator *CYCLINA2;1* (*CYCA2;1*) caused the exaggerated ethylene-induced leaf movement of *EHY-D*. Mathematical analyses indicated that besides promoting cell expansion, ethylene can also attenuate the amplitude of hyponasty by affecting differential cell proliferation in the petiole of wild-type plants. We suggest that this occurs through ethylene-dependent effects on *CYCA2;1* levels, activity or sensitivity in petioles of wild-type plants. The here observed ethylene-mediated transcriptional regulation of *CYCA2;1* could contribute to this. In *EHY-D* however, ethylene-mediated effects on cell proliferation are overruled by ectopic *CYCA2;1* overexpression, which consequently results in enhanced hyponasty, in accordance with the predictions of our model. Correspondingly, *cyca2;1* knock-out lines where ethylene cannot affect *CYCA2;1*-mediated cell proliferation also exhibited enhanced hyponasty. Our data therefore describe a novel mechanism how hyponastic growth is kept within limits, through a novel bipartite role for ethylene; Within the same organ ethylene initiates hyponastic growth by promoting cell elongation, while simultaneously attenuating the response by regulation of A2-type CYCLIN-mediated cell proliferation.
Results

Isolation and cloning of Enhanced Hyponasty-D (EHY-D)

To identify novel genetic components that control hyponastic growth, we conducted a forward genetic screen using a population of 35S activation-tagged Columbia (Col) plants (Weigel et al., 2000). 17,500 plants were screened for their hyponastic response under 6 h of ethylene and low light treatment. The screen yielded 18 candidates with aberrant petiole angle (Polko et al., 2012b, 2013). Among the isolated lines was Enhanced Hyponasty-D (EHY-D), which showed an initial petiole angle similar to wild-type (20.5 ± 1.4 and 22.5 ± 1.4 respectively, Supplemental Table S1) and an exaggerated response to ethylene (Fig. 1A,C) and low light (Fig. 1C). No other apparent visual differences were observed (Fig. 1B). The enhanced hyponasty phenotype was confirmed by quantitative analysis of leaf movement kinetics using a time-lapse digital camera setup (Fig. 1D-F). In addition, high temperature also resulted in an enhanced response (Fig. 1C,F), suggesting that a general genetic determinant of hyponastic growth is affected in EHY-D.

The phenotype co-segregated with the transgene in a 3:1 ratio (77.3 ± 2.0% BASTA resistant), indicating that EHY-D has a single T-DNA integration. Sequencing of the T-DNA flanking borders after Thermal Asymmetric Interlaced-PCR (Liu et al., 1995) revealed that the insertion is on chromosome 5 in the intergenic region between ETHYLENE RESPONSE FACTOR/APETALA2 (ERF/AP2 transcription factor subfamily B-6; SHINE3) (SHN3; At5g25390; Nakano et al., 2006) and the mitotic check-point regulator CYCLINA2;1 (CYCA2;1; At5g25380) (Fig. 1G).

Genes causal for observed phenotypes are often flanking or in the direct vicinity of the T-DNA insertion site (Weigel et al., 2000). Therefore, we quantified the relative transcript levels of the genes within a 15 kb region up- and downstream of the T-DNA integration site by qRT-PCR under control conditions and after application of ethylene. Some of the tested genes where mildly upregulated after 3h of ethylene treatment compared to control conditions in wild-type Col (Table I). This included EIN3-BINDING F-BOX PROTEIN 2 (EBF2) that was previously shown to be ethylene inducible (Potuschack et al., 2003). In EHY-D, only the two genes directly flanking the T-DNA insertion border (SHN3 and CYCA2;1) were overexpressed compared to wild-type Col, and this was true under both control and ethylene conditions (Table I). This suggests that one of these genes is causal for the exaggerated hyponastic growth phenotype of EHY-D.

Overexpression of CYCA2;1 mimics the EHY-D hyponastic growth phenotype

SHN3 encodes a member of the ETHYLENE RESPONSE FACTOR (ERF) family. ERFs control many developmental and physiological processes, including several ethylene-mediated responses (Nakano et al., 2006). Using overexpression lines in the Wassilewskija (Ws) (Aharoni et al., 2004) and Col backgrounds (isolated from the collection described in Weiste et al. (2007)), we tested if SHN3 overexpression could be responsible for the observed exaggerated hyponasty in EHY-D. However, overexpression of SHN3 did not result in enhanced hyponastic responses in neither the Col nor Ws background (Supplemental Fig. S1). This indicates that SHN3 overexpression in EHY-D is not causing its exaggerated hyponastic growth phenotype.
CYCA2;1, the highly upregulated gene directly flanking the EHY-D locus (Fig. 1G), belongs to a small gene family of G2-to-M cell cycle regulators (Yoshizumi et al., 2006; Vanneste et al., 2011). To test if CYCA2;1 overexpression could explain the EHY-D phenotype, we generated 35S::CYCA2;1 plants. As observed in EHY-D, hyponastic growth was enhanced in four independent CYCA2;1 overexpression lines (Fig. 2A-C, Supplemental Fig. S2; S3A). The differences in hyponastic growth response between the independent lines was positively correlated with the respective CYCA2;1 expression levels (Supplemental Fig. S3B). Moreover, a mutant defective in the conserved and specific A2-type CYCLIN repressor INCREASED LEVEL OF POLYPLOIDY1-2 (ilp1-2), which results in enhanced expression of all A2-type CYCLIN family members (Yoshizumi et al., 2006), also showed exaggerated hyponastic growth under ethylene exposure (Fig. 2D). This response was comparable to EHY-D and 35S::CYCA2;1 lines. Consistently, the ILP1-D activation-tagged line with decreased expression of all four A2-type CYCLINs (Yoshizumi et al., 2006) showed reduced hyponastic growth (Fig. 2E).

Transcription of other A2-type CYCLIN family members (Vandepoele et al., 2002) and several other cell cycle marker genes (Yoshizumi et al., 2006) was not distinctly affected in whole-petioles of EHY-D (Fig. 2F) in neither control nor ethylene conditions. Taken together, these data demonstrate that overexpression of CYCA2;1 is sufficient to explain the EHY-D hyponastic growth phenotype.

Surprisingly, when we assayed the requirement of functional CYCA2;1 for hyponastic growth we found that two independent knock-out alleles of cyca2;1 also showed an exaggerated response to ethylene, low light and high temperature treatment (Fig. 2G,H; Supplemental Fig. S4A-D; Supplemental Table S1). Because it has been reported that reduced CYCA2;2 expression in erecta loss-of-function mutants can be compensated for by ectopic upregulation of CYCA2;3 (Pillitteri et al., 2007), we tested for compensatory upregulation of other A2-type CYCLINs in cyca2;1 mutant petioles by qRT-PCR. However, our qRT-PCR experiments did not reveal ectopic upregulation of CYCA2;2, CYCA2;3 nor CYCA2;4 in petiole tissues of the cyca2;1-2 mutant (Supplemental Fig. S4E). Similarly to EHY-D (Fig. 2F), these genes were also not affected in the 35S::CYCA2;1 line (Supplemental Fig. S4F). Therefore, compensatory transcriptional upregulation of other A2-type CYCLINs in the petiole probably cannot explain the enhanced hyponastic growth response of cyca2;1 mutants. However, from these data we cannot exclude that changes in spatio-temporal expression of other A2-type CYCLINs affect the hyponastic growth response.

Therefore, we also analyzed cyca2;2-1, cyca2;3-1 and cyca2;4-1 insertion mutants (Vanneste et al., 2011). In contrast to the cyca2;1 mutants (Fig 2G,H), these single mutants did not show an altered hyponastic growth phenotype in response to ethylene (Supplemental Fig. S5A-C). When combined with other mutations in the A2-type CYCLIN family, the exaggerated cyca2;1 hyponastic growth was however lost and was in some cases even lower than wild-type (Supplemental Fig. S5D-L), suggesting that misregulation of other CYCA2s could be related to the cyca2;1 mutant phenotype. This is consistent with the results of ILP1-D where all A2-type CYCLINs were transcriptionally down regulated (Fig 2E) and demonstrates involvement of other A2-type CYCLINs in the control of hyponastic growth. To address this, we analysed the hyponastic growth of a CYCA2;2 overexpression line. Similarly to the EHY-D and 35S::CYCA2;1 lines, the 35S::CYCA2;2 line also showed enhanced hyponasty in response to ethylene and low light, but not in response to high
temperature (Supplemental Fig. S5M-O). In addition, also the initiation of the response to low light and high temperatures was delayed, suggesting that besides CYCA2;1 other A2-type CYCLINs are also biochemically competent in modifying hyponastic growth responses.

**Ethylene suppresses expression of mitotic genes in the petiole**

To determine if CYCA2;1 is specifically involved in hyponasty or is modifying a general component in ethylene-mediated growth responses, we assayed ethylene-dependent inhibition of hypocotyl elongation in dark-grown seedlings (Guzman and Ecker, 1990). No differences in elongation were detected in EHY-D, 35S::CYCA2;1 and cyca2;1-2 compared to wild-type (Fig. 3A) in the presence of increasing concentrations of the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC). This indicates that CYCA2;1 levels do not affect ethylene sensitivity of the hypocotyls.

Additionally, ethylene release from vegetative rosettes of these lines was similar to that of the wild-type (0.65 ± 0.13 in EHY-D, 0.64 ± 0.09 in 35S::CYCA2;1 and 0.52 ± 0.06 in cyca2;1-2, compared to 0.65 ± 0.13 nl g FW⁻¹ h⁻¹ in wild-type). These results suggest that CYCA2;1 regulation does not modify general ethylene-mediated growth responses, but may have a specific role in hyponastic growth.

To evaluate if ethylene affects A2-type CYCLINs expression in the petioles, we first assayed promoter activity of the four A2-type CYCLINs by promoter::GUS analyses (Burssens et al., 2010; Vanneste et al., 2011). All A2–type CYCLIN promoters were active in meristematic tissues at the rosette centre and were mainly, but not exclusively, localized in vascular tissues (Supplemental Fig. S6A,B). Observations of radial sections of whole petioles (Supplemental Fig. S7A), detailed analysis of proximal and distal petiole tissues (relative to the meristem) (Supplemental Fig. S7B) and transverse sections (Fig. 3B) of histochemically stained petioles were variable but showed overall that ethylene results in transcriptional repression of A2-type CYCLINs after 6 h of ethylene treatment, despite that the GUS protein is relatively stable and transcriptional down regulation was not yet measurable after 3 h by qRT-PCR (Fig. 2F). These results suggest that ethylene suppresses A2-type CYCLIN expression in Arabidopsis petioles.

To corroborate these findings based on promoter::GUS analyse, we analysed A2-type CYCLIN expression in micro-dissected fragments of wild-type petioles (Fig. 3C) by qRT-PCR. This revealed that CYCA2;2, CYCA2;3 and CYCA2;4 are transcriptionally down regulated after prolonged (6 h) ethylene treatment in all four fragments (Fig. 3D). Notably, CYCA2;1 was also generally down regulated including in the proximal-abaxial section (fragment 1, Fig. 3C,D), but appeared modestly up regulated at the proximal-adaxial side (Fig. 3D).

Because A2-type CYCLINs control a cell cycle check-point upstream of the expression of mitotic regulators such as B-type CYCLINs (Vanneste et al., 2011), we anticipated and confirmed that ethylene also represses expression of the mitotic CYCLIN, CYCB1;1 in these tissues (Fig. 3E).

These results are consistent with a suppression of proliferation by ethylene in petiole tissues.

**Endoreduplication cannot explain exaggerated hyponastic growth in EHY-D and 35S::CYCA2;1**
A2-type CYCLINs have been implicated in the control of local cell cycle progression to fine-tune development (Vanneste et al., 2011). More specifically, their expression levels can affect the local balance between cell proliferation and endoreduplication, a process of consecutive rounds of DNA replication without mitosis (Yu et al., 2003; Imai et al., 2006; Yoshizumi et al., 2006) that has been associated with cells that have an increased capacity for elongation (Cheniclet et al., 2005; Roeder et al., 2010). Despite previous reports on ethylene-related changes in endoreduplication in hypocotyls (Dan et al., 2003; Gendreau et al., 1999), we did not find significant ethylene-dependent differences in ploidy levels in micro-dissected proximal and distal fragments of wild-type petioles (Supplemental Fig. S8A). However, we did observe slightly different ploidy levels between distal and proximal regions of the petiole (Supplemental Fig. S8A), as well as small but significant differences in the 2n, 4n, 16n and 32n classes between wild-type and EHY-D (p=0.041, p=0.036, p=0.007, p=0.05, respectively; Supplemental Fig. S8B). However, no significant differences were detected between wild-type and 35S::CYCA2;1 (except for the 4n class p=0.025) (Supplemental Fig. S8B), making it unlikely that the exaggerated hyponastic growth of both CYCA2;1-overexpressing lines (EHY-D and 35S::CYCA2;1) can be explained by changes in ploidy levels. Moreover, a dominant negative CYCLIN-DEPENDENT KINASE B1-1 (CDKB1;1) overexpressing line with enhanced ploidy levels in aerial organs (Boudolf et al., 2009) showed ethylene-induced hyponastic growth that was indistinguishable from the wild-type (Supplemental Fig. S8C). Together, these data argue against a major role of endoreduplication in the exaggerated hyponastic growth in EHY-D.

Ectopic overexpression of CYCA2;1 comprises ethylene-mediated differential cell proliferation during hyponastic growth

We examined if enhanced hyponasty in 35S::CYCA2;1 is due to enhanced cell expansion in the petiole compared to the wild-type. Measurements of epidermal cell lengths revealed that significant cell expansion in 35S::CYCA2;1 under ethylene treatment occurs in a ~2 mm epidermal zone at the proximal abaxial side of the petiole (Fig. 4A,B). The pattern of changes in cell size strongly resembles the pattern previously observed in wild-type Arabidopsis Col plants (Fig. 4C,D; Polko et al., 2012a, Rauf et al., 2013). Moreover, 35S::CYCA2;1 showed a similar ethylene-induced cortical microtubule (CMT) reorientation as described previously for wild-type plants (Polko et al., 2012a) (Supplemental Fig. S9A-G). Although this suggests that the exaggerated hyponastic growth in 35S::CYCA2;1 is not due to differences in cell expansion compared to wild-type, this cannot be concluded without taking cell proliferation into account (see Supplemental Text S1). However, due to experimental constraints, cell division rates cannot be derived from empirical in vivo cell length measurements (see Supplemental Text S1). This is because both time-lapse imaging of cell division as well as destructive measurements directly interfere with the hyponastic response itself. Nevertheless, the dynamic petiole shape and static cell size distributions as observed from epidermal-imprints (Fig. 4A-D), together provided sufficient information to allow for a mathematical analysis that indirectly estimates the contribution of cell divisions within the petiole. Using such a mathematical approach, we calculated relative division rates between abaxial vs. adaxial cells, which is sufficient to describe its role in petiole hyponasty. Theoretical details can be found in the Materials and Methods and Supplemental Text S2 & S3. The mathematical analysis showed that in wild-type
plants ethylene treatment strongly increased the bias towards adaxial cell proliferation in the proximal region of the petiole, with adaxial cell division rates during ethylene treatment being up to 80% higher than abaxial cell division rates (Fig. 4E,F). This indicates that in this region ethylene triggers either increased adaxial cell proliferation or decreased abaxial cell proliferation. Our qRT-PCR and GUS analysis showed that ethylene in general suppresses cell proliferation markers (Fig. 3B,D, S6, S7). The most likely scenario would therefore be a decrease in cell proliferation. The analysis predicts that a local reduction of cell proliferation rate in a ~2 mm-long epidermal cell zone at the proximal part of the abaxial side of the petiole is required to match the measured amplitude of hyponastic growth in wild-type. This is in accordance with Polko et al. (2012a), where a comparable analysis showed that under the assumption that abaxial and adaxial cell proliferation rates are equal, the observed changes in abaxial cell elongation rates during ethylene treatment result in a larger hyponastic response in comparison to what was experimentally observed. We observed that the 35S::CYCA2;1 line was lacking such increased bias towards adaxial cell proliferation after ethylene treatment. Instead, in this line the cell proliferation profile in ethylene was estimated to be highly similar to the cell proliferation profile in the untreated controls (Fig. 4E,F). In other words, cell proliferation rates in 35S::CYCA2;1 are predicted to be comparable between control and ethylene treatment, while in the wild-type ethylene exposure represses abaxial cell divisions relative to adaxial cell divisions.

Together, this suggests that ethylene controls differential cell proliferation in the petiole, thereby affecting the amplitude of ethylene-induced hyponastic growth. Our calculations indicate that ethylene causes enhanced abaxial cell elongation leading to hyponastic growth, while at the same time suppressing proliferation, thus attenuating hyponasty. In 35S::CYCA2;1 this secondary control mechanism is overruled, leading to exaggerated hyponastic growth.

In silico modelling of hyponastic growth corroborates that absence of differential cell proliferation leads to exaggerated hyponasty

Tissue growth can be described by the combination of cell expansion and cell division. However, cell divisions, as opposed to cell elongation, do not immediately generate volumetric tissue growth (only extra cells). Therefore, divisions can only have an indirect effect on tissue growth (Harashima & Schnittger, 2010). The effect of cell division on tissue growth depends on the specific relationship between cell size and cell expansion, as is discussed in Supplemental Text S4. Our mathematical analysis on petiole epidermal cell lengths indicated that the amplitude of ethylene-induced hyponastic growth is mediated by a differential regulation of cell division and that the enhanced hyponastic growth response in 35S::CYCA2;1 is correlated with an absence of reduction in cell division (Fig. 4E,F), suggesting that CYCA2;1 has a role in the mechanism that mediates the hyponastic growth response specifically via reduced abaxial cell proliferation. To further explore the relationship between (abaxial) cell division and the hyponastic growth response, we developed an in silico model of the Arabidopsis petiole. With the model we simulated ethylene-induced hyponastic growth for different scenarios of cell expansion: linear, exponential, logistic and logarithmic growth and division rates. Since it has been shown that the epidermal cell layer is sufficient to drive and restrict plant growth (Savaldi-Goldstein et al., 2007), we modelled only the epidermal layers of the
abaxial and adaxial sides of the petiole (Fig 5A). The experimental data showed that cell size increases along the petiole (Fig. 4A,B). We simplified the growth and cell-cycle dynamics by in first instance assuming that, apart from the adaxial-abaxial differences, the petiole is spatially homogenous, i.e. that cell expansion and division are not influenced by their proximal-distal position in the petiole (for details on the in silico modelling see Materials and Methods and Supplemental Text S5).

Because the precise relationship between cell size and cell expansion dynamics is not well established (see Supplemental Text S5), we simulated hyponastic growth of the petiole for different possible scenarios of cell expansion, namely linear, exponential, logistic and logarithmic, and combined this with different cell division scenarios.

A first round of simulations assessed the possible effect of reduced cell division in the proximal abaxial region on petiole shape for the different possible cell expansion scenarios, other than an increase in abaxial cell expansion due to ethylene treatment, and analysed whether a reduction in cell proliferation (ranging from 0-100%) within that region could indeed attenuate the hyponastic response that would be expected without reduction in abaxial cell proliferation (Fig 5B). These simulations support the idea that reduction in abaxial cell proliferation leads to reduction in hyponastic petiole curvature, except when cell expansion is exponential. As explained in Supplemental Text S4 however, exponential cell expansion implies that the occurrence of cell divisions has no influence whatsoever on the tissue growth, which is an unrealistic scenario. These simulations thus indicate that a decrease in abaxial cell division is expected to reduce hyponastic growth.

Next, we explored the role of cell expansion arrest during cell division. In the simulations described above, we did not take into account that the cell division event itself could directly affect the cell expansion. It is very likely, however, that cell expansion is arrested for a certain amount of time when the cell goes through mitosis (Beemster & Baskin, 1998; Grieneisen et al., 2007). Fig. 5C shows that prolonged periods of arrest in expansion during mitosis can counter-balance the effect of reduced abaxial cell division on petiole curvature, but only for a duration of the expansion arrest that is unrealistically long.

Next, we used this model to evaluate the impact of misregulated cell proliferation, as expected for overexpression or mutation of CYCA2;1 (Fig. 5D-G). To capture the complete petiole shape, we used the observation that cell division and elongation are limited to the proximal 3 mm of the petiole (Fig. 4A,B). The shape of the distal part of the petiole is therefore considered conserved over the period of the experiment (see Supplemental Text S5). For all three genetic backgrounds (wild-type, 35S::CYCA2;1 and cyc2;1), we parameterized that ethylene treatment increases abaxial cell elongation (Fig. 4A,B) and that mitosis causes a 1h-arrest in cell expansion. Following our hypothesis and the simulations described above, we assumed that in the wild-type abaxial cell division decreases during ethylene-induced hyponastic growth (Fig 5E). Alternatively, we modelled genetic backgrounds that lack such differences between abaxial and adaxial cell division by setting overall cell division rates to be constitutively higher and constitutively lower, reflecting scenarios of overexpression and mutation of CYCA2;1, respectively (Fig 5F,G). As was observed in the experimental measurements, the simulations result in an increased hyponastic growth response for
both 35S::CYCA2;1 and, to a slightly lesser extent, cyca2;1, except under the unrealistic scenario of exponential growth (Fig. 5D).

Taken together, our in silico model shows that reduced abaxial cell division decreases the amplitude of hyponastic growth. Furthermore, it demonstrates that when this mechanism is impaired by either constitutive CYCA2;1 overexpression or by a knock-out mutation, the hyponastic growth response becomes exaggerated, as was experimentally observed in both 35S::CYCA2;1 and cyca2;1 (Fig. 2A,G,H).
Hyponastic growth is an adaptive response by which plants cope with adverse environmental conditions. The response is controlled by complex interactions between various phytohormones. However, since hyponastic growth induced by various independent environmental stimuli is highly similar in kinetics and amplitude, the signalling mechanisms likely converge downstream on specific functional molecular components that control the response (Van Zanten et al., 2010a). We aimed to identify novel molecular hyponastic growth regulators and isolated EHY-D, which has exaggerated amplitudes of leaf movement upon induction by ethylene, low light intensity and high temperatures. Because EHY-D exhibited an enhanced response to each treatment investigated, the insertion likely affects a general downstream determinant of hyponasty. Our studies show that the core-cell cycle regulator CYCA2;1 was overexpressed in EHY-D. Several independent A2-type CYCLIN overexpression lines and mutants showed consistently altered hyponastic growth phenotypes (Fig. 2, Supplemental Fig. S3-5), indicating that A2-type CYCLINs are important determinants of the hyponastic response.

Our results suggest that A2-type CYCLINs operate in a specific branch of ethylene signalling that affects differential growth, but not hypocotyl elongation. We found that prolonged (6 h) ethylene treatment results in down regulation of A2-type CYCLINs in the petiole (Fig. 3B,D). This down regulation is initiated at least 3 h after start of ethylene treatment because up to this time-point A2-type CYCLINs transcription was unaffected (Fig. 2A,F). Since hyponastic growth is induced already within the first hour after ethylene application, transcriptional control of A2-type CYCLINs likely does not control the induction of hyponastic growth, which occurs within the first 1-2 hours after perception of ethylene.

The promoter region of CYCA2;1 contains eight Ethylene Response Factor-binding Ethylene Responsive Elements (EREs) (Richard et al., 2001), implying that ethylene could control its transcription directly through ERF transcription factors that have the ERE as their promoter targets.

Hyponastic growth does not depend on ploidy levels

A-type CYCLINs are expressed at the S-to-M transition of the mitotic cell cycle, prior to activation of B-type CYCLINs (Inzé and De Veylder, 2006) and are rate-limiting factors for entry in the mitotic cell phase (Burssens et al., 2000; Dewitte and Murray, 2003; Yu et al., 2003; Vanneste et al., 2011). Down regulation of CYCA2 levels causes a shift towards endoreduplication (Imai et al., Yoshizumi et al., Vanneste et al., 2011) and is associated with developmentally controlled cell cycle exit (Vanneste et al 2011). This process is generally associated with differentiating cells undergoing cell expansion (Sugimoto-Shirasu and Roberts, 2003), e.g. in Brassica oleracea petals (Kudo and Kimura, 2002). Consistently, ethylene has been shown to induce endoreduplication events in hypocotyls of Cucumis sativus and Arabidopsis (Dan et al., 2003; Gendreau et al., 1999). In our study, however, neither CYCA2;1 overexpression nor ethylene application strongly affected ploidy levels in Arabidopsis petioles, which suggests that ethylene-induced hyponastic petiole growth is not regulated by CYCA2-mediated effects on the endocycle. This is in agreement with a previous study showing that petiole growth is independent of changes in ploidy levels (Kozuka et al., 2005). Possibly, the occurrence and
role of endoreduplication in organ growth is less pronounced in petioles of mature plants than in hypocotyls of very young seedlings.

**Differential cell proliferation can control hyponastic growth amplitudes**

Our finding that CYCA2;1 overexpression or mutation causes an exaggerated hyponastic growth response is difficult to explain intuitively. Therefore, we developed an *in silico* model based on experimentally determined parameters, incorporating the effect of cell elongation and cell proliferation on petiole shape. It was critically important that the model predicted that a lack of differential cell proliferation between ab- and adaxial petiole sides results in stronger hyponastic growth responses.

By combining cell length data and petiole shape we were able to assess the influence of ethylene treatment and constitutive CYCA2;1 expression on relative cell proliferation rates during hyponastic growth. This mathematical analysis showed that in wild-type petioles adaxial cell proliferation increases relative to abaxial cell proliferation during ethylene treatment. This can be caused by an increase in adaxial cell proliferation, a decrease in abaxial cell proliferation or a combination of both. The scenario of decreased abaxial cell proliferation is the most likely explanation for the observed effect on hyponastic growth in the wild-type, as this is in line with the observed down regulation of A2-type CYCLINs expression following ethylene treatment. This is in accordance with a previous study indicating that ethylene can arrest the cell cycle by directly affecting core-cell cycle components (Skirycz *et al.*, 2011). Together, these data, combined with our mathematical analyses and the *in silico* model, suggest that the control of the amplitude of ethylene-induced hyponastic growth relies on a dual mechanism. Ethylene enhances cell elongation along the abaxial side of the petiole in wild-type plants, providing the tissue growth required for the upward movement of the petiole, while also down regulating CYCA2;1 and CYCB1;1 expression, conceivably in a differential manner (see below), reducing abaxial cell proliferation. This reduced cell proliferation counteracts the effects of cell elongation, thereby attenuating the amplitude of the hyponastic response. When CYCA2;1 is constitutively expressed, no differential inhibition of cell proliferation occurs, leading to an exaggerated hyponastic response. Our *in silico* model of hyponastic growth provides a proof-of-concept for this mechanism. Importantly, in addition to confirming the exaggerated response when CYCA2;1 is constitutively expressed, the model also predicts that the same mechanism results in exaggerated hyponastic growth response when no CYCA2;1 is present. This was experimentally observed using *cyca2;1* knock-out lines, which indeed show exaggerated hyponasty under ethylene treatment (Fig. 2G-H).

The exact molecular mechanism by which ethylene installs differential cell proliferation between the abaxial and adaxial petiole side in ethylene-treated wild type plants remains to be elucidated. Our work suggests that CYCA2;1 is critically involved. In this context it is essential to consider the central role of distinct Cyclin Dependent Kinases (CDK) in complex with CYCLINs in controlling cell cycle check-points during cell proliferation. Besides the association with distinct CYCLINs whose levels are controlled at the level of transcription and protein stability, CDK activity is further fine-tuned via interaction with proteins such as Kip-Related Proteins and regulatory phosphorylation events (Inze &
De Veylder, 2006; Polyn et al., 2015). Even if such components are involved, it remains unknown how the abaxial versus adaxial differentiation comes about and this remains an important question for future studies.

The effect of ethylene on cell proliferation is complex and largely depends on the tissue context. On the one hand, ethylene was found to stimulate proliferation in the Arabidopsis root stem cell niche (Ortega-Martinez et al., 2007), in submergence-induced adventitious root growth in rice (Lorbiecke & Sauter, 1999), and in subsidiary cells of cucumber hypocotyls and vascular tissues (Love et al., 2009; Etchells et al., 2012). On the other hand, in developing leaves ethylene was found to suppress proliferation during mild osmotic stress (Skyricz et al., 2011), alike the suppression in petioles presented in this work. Interestingly, this osmotic stress-induced cell cycle arrest is associated with regulation of CDKA;1 activity that does not involve ETHYLENE INSENSITIVE 3 (EIN3)-mediated transcriptional changes (Skyricz et al., 2011), suggesting that the effect of ethylene on differential regulation of proliferation in the petioles could involve non-transcriptional regulation. Consistently, we found that down regulation of \( A2\text{-type CYCLINs} \) in the petiole does not occur within 3h of ethylene treatment (Fig. 2), while hyponastic growth is induced within the first hour after ethylene application.

The bipartite role for ethylene in hyponastic response

In conclusion, we propose a dual role for ethylene in the mechanism regulating hyponastic growth. Ethylene i) induces cell elongation in the abaxial petiole epidermal cells to power the upward leaf movement and ii) inhibits the mitotic cell cycle, likely in part by affecting \( CYCA2;1 \) expression, in the same tissue. An \textit{in silico} model confirmed that such a mechanism can explain the observed exaggerated hyponastic growth in both 35S::\( CYCA2;1 \) and \( cyca2;1 \) null-mutants. The dual role for ethylene found in this work adds to an increasing number of studies that indicate both growth stimulatory and inhibitory roles for ethylene in plant development, abandoning the classic idea of ethylene simply being a growth inhibitor. The hormone rather inhibits or stimulates growth in a subtle manner that integrates information from the environment together with developmental state and cellular identity of a tissue/organ (reviewed in Pierik et al., 2006)). This mechanism controls the magnitude of ethylene-induced hyponastic leaf movement in an effort to optimize plant performance under stressful conditions.
Materials and Methods

Plant material growth conditions

Arabidopsis thaliana lines were from the Nottingham Arabidopsis Stock centre (IDs between brackets) or were a kind gift of authors. Col (N1092), activation-tagged lines (Weigel et al., 2000; N21991, N23153), Ilp1-2, ILP1-1D (Yoshizumi et al., 2006), 35S::CDKB1;1.N161 line 9.2 (Boudolf et al., 2009), 35S::SHN3 (Aharoni et al., 2004), D-Box-CYCB1;1::GUS (Colon-Carmona et al., 1999; Wildwater et al., 2005), CYCA2;1::GUS (Burssens et al., 2000; Vanneste et al., 2011), CYCA2;2::GUS, CYCA2;3::GUS and CYCA2;4::GUS (Vanneste et al., 2011). The 35S::TUA6:GFP (Ueda et al., 1999) line was a gift from Douglas Muench (University of Calgary, Canada). cyca2;2-1 (SALK_121077; Yoshizumi et al., 2006), and cyca2;1-2 (SALK_123348; Vanneste et al., 2011), cyca2;2-1 (GABI_120D03), cyca2;3-1 (SALK_092515), cyca2;4-1 (SALK_070301). Double and triple mutants are based on crosses between these lines and are described in Vanneste et al. (2011). All alleles are representative knock-outs.

Isolation of 35S::At3g25390 from the AtTOTF-Ex ERF ectopic expression library (Weiste et al., 2007) is described in Supplemental Methods S1. This line had a 11.7 ± 0.2 times higher expression of SHN3 than wild-type, as determined by qRT-PCR.

Seeds were stratified at 4 °C for 4 d, sown on fertilized mixture of soil and perlite and grown at 20 °C, 70 % (v/v) RH, 200 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) (9 h photoperiod) as described earlier (Millenaar et al., 2005). 30 d old plants in stage 3.9 (Boyes et al., 2001) were used for all experiments, except for the hypocotyl elongation assay (below). One day before the start of the experiments plants were transferred to the experimental setup with similar conditions to the growth chambers (Microclima 1750 growth cabinet; Snijders Scientific, Tilburg, The Netherlands). To rule out effects of diurnal and circadian leaf movements, all treatments commenced 1.5h after the start of the photoperiod.

To generate 35S::CYCA2;1 and 35S::CYCA2;2 plants, full-length cDNA was cloned through GATEWAY technology (Invitrogen, Gaithersburg, MD, USA) in pDONR221 and subcloned into pK2GW7.0 and pKGWFS7.0 respectively (Karimi et al., 2002). 35S::CYCA2;1 line Hmz B (Supplemental Fig. S2) was used in all experiments involving CYCA2;1 overexpression.

Real-time reverse transcriptase-PCR and Histochemical β-glucuronidase staining

Real-time reverse transcriptase-PCR was conducted as described in Millenaar et al. (2005) and is described in detail in the Supplemental Methods S1. Primers are shown in Supplemental Table S2 and in Mariconti et al. (2002), Richard et al. (2001), Vanneste et al. (2011) and Yoshizumi et al. (2006).

For histochemical β-glucuronidase staining, tissues were harvested and placed briefly in 90% ice-cold acetone and subsequently fixed and vacuum infiltrated with 10 mM MES, 0.3 M mannitol, 0.3% formaldehyde, for 45 min. Tissues were rinsed in 100 mM Buffer (50 mM NaHPO₄ + 50 mM Na₂HPO₄; pH 7.2). The histochemical reaction was performed by incubation in 1 mM X-Gluc (5-bromo-4-chloro-3-indolyl β-D-glucoronide) in DMSO for 24 h at 37 °C. The tissues were cleared in
ethanol series of increasing concentrations (5% to 90%) and were either hand-sectioned and directly observed or first embedded in Technovit 7100 (Kulzer, Germany) and dissected using a microtome. The resulting 200 µm sections were observed and photographed with an Olympus BX50 WI mounted with an Olympus DP 70 camera.

**Ethylene, low light and high temperature treatments**

Ethylene (Hoek Loos, Amsterdam, The Netherlands) was applied to saturating (Polko et al., 2012a) concentrations (1.5 μl l⁻¹; except for the hyponastic growth kinetics experiment, see below), in continuous flow-through and then vented away. The concentration was regularly checked by gas chromatograph analysis. The control treatment was done in the same experimental cabinet. For leaf movement kinetics analysis, 5 μl l⁻¹ ethylene was mixed with humidified air (70% (v/v) and applied to glass cuvettes containing one plant each at a flow-rate of 75 l h⁻¹ as described in Millenaar et al. (2005).

Low light intensity was induced by decreasing PAR-level from 200 μmol m⁻² s⁻¹ to 20 μmol m⁻² s⁻¹ by switching off lamps and by covering the plants with spectrally neutral shade cloth. This did not change light quality (checked with a LI-COR 1800 spectro-radiometer (LI-COR, Lincoln, NE, USA). Induction of high temperature was accomplished by moderating the program of the used growth cabinet. The 30°C threshold was reached after 22 minutes; 38°C was reached after 49 minutes.

**Genetic screen and cloning of EHY-D**

For details on the genetic screen that identified EHY-D, see Polko et al. (2012b). To facilitate easy and fast screening, we first checked if wild-type plants were able to exhibit a normal low light-induced hyponastic response after 6h ethylene treatment and an overnight recovery. Ethylene-induced hyponastic growth was, as expected, quickly reversed by removing the ethylene source (Millenaar et al., 2005) and this treatment did not interfere with low light-induced hyponasty in the subsequent photoperiod (next day) (Supplemental Fig. S10). In total, we screened 17,500 individual Cauliflower Mosaic Virus (CaMV) 35S enhancer (activation) tagged (Weigel et al., 2000) vegetative plants in developmental stage 3.7 (Boyes et al., 2001). The plants were visually monitored for i) petiole angle after 6 h of ethylene treatment and after overnight recovery ii) the petiole angle after 6h low light treatment. To check the number of inserts, crosses were made between wild-type and the glufosinate ammonium (BASTA)-resistant EHY-D. Self-pollinated F2 progeny seeds were subjected to BASTA selection on MS agar-plates containing 8 g l⁻¹ plant agar (Duchefa, Brussel, Belgium), 0.22 g l⁻¹ Murashige-Skoog (Duchefa) and 50 μg ml⁻¹ DL-Glufosinate ammonium (BASTA / DL-phosphinothricin; Duchefa). After three weeks survival ratios were scored. TAIL-PCR was conducted to identify the T-DNA locus in EHY-D as described by Liu et al., (1995). For details see Supplemental Materials and Methods S1.

**Petiole angle measurements**

Petiole angle kinetics was measured using an automated time-lapse camera system as described in Millenaar et al. (2005). Plants were placed in glass cuvettes with the petiole of study perpendicular to the axis of the camera. To facilitate measurement, leaves obscuring the petiole base were removed.
and an orange paint droplet (Decofin Universal; Apeldoorn, The Netherlands) was used to mark the petiole/lamina junction. This did not affect the response (data not shown). Digital images of two petioles per plant were taken every 10 min. Angles were measured between the marked point at the petiole/lamina junction and a fixed proximal point of the petiole, relative to the horizontal, by using KS400 (Version 3.0) software package (Carl Zeiss Vision, Hallbergmoos, Germany) and a custom made macro. To enable continuous photography over the 24 h experimental period, no dark period was included during the experiments.

Plants used for measurements at fixed time-points were manually photographed from the side. Angles were measured using ImageJ Software (Abramoff et al., 2004). Before further analysis, two petioles per plant were measured and an average was calculated. Statistical significance-levels were determined using type-2 2-tailed Student’s T-Test.

To rule out diurnal- and/or circadian effects on petiole movement, a pair-wise subtraction was performed on hyponastic growth data. Differential petiole angle describes a difference between the angle in control vs. experimental conditions at each time point (Benschop et al., 2007). The new standard error for the differential response was calculated by taking the square root from the summation of the two squared standard errors. Initial petiole angles at t=0 h of the A2-type CYCLIN-related lines are shown in Supplemental Table S1.

**Cell length measurements and CMT visualisation**

Cell length measurement of epidermal imprints of 1 cm-long petioles and visualization of arrangement of cortical microtubules (CMTs) were performed as described in Polko et al. (2012a). Cell lengths were quantified using a custom made macro in KS400 software (Zeiss, Halbergmoos, Germany). Each cell was assigned to a 200 µm class, according to its position relative to the most proximal part of the petiole.

To visualize microtubules in the 35S::CYCA2;1 background, we crossed this line with 35S::TUA6:GFP (Ueda et al., 1999). After 5 to 10h of the ethylene/control treatment CMTs of petiole epidermal cells were visualized using an inverted confocal laser-scanning microscope (Leica CS SPII, 63x C-apochromat objective, excitation wavelength of 488 nm, collecting at 505-530 nm for GFP emission). Petioles were divided in quadrants depending on their distance from the base and the ab- and adaxial sides were observed separately. Only CMT areas at least twice as long as cell width were taken into account and grouped in categories relatively to the long cell axis: transverse (0°), oblique 30°, oblique 60°, longitudinal (90°) and randomly oriented, according to Himmelspach and Nick (2001).

**Ploidy measurements**

For ploidy measurements, two petioles or petiole segments of at least two plants (replicas) were snap frozen in liquid nitrogen, ground in extraction buffer (CyStain UV precise buffer P; Partec, Münster, Germany), diluted in a Staining buffer (CyStain UV precise buffer P; Partec) containing 4',6-diamidino-2-phenylindole, and analyzed on a Cytoflow ML flow-cytometer (Partec).

**Hypocotyl elongation assay and ethylene release measurements**
Hypocotyl elongation assay was conducted as described in Van Zanten et al. (2010b). Sterilized seeds were sown on petri dishes containing Murashige-Skoog-enriched plant agar (4 g l\(^{-1}\) plant-agar (Duchefa), 0.22 g l\(^{-1}\) MS (Duchefa), and different concentrations of 1-aminocyclopropane-1-carboxylic acid (ACC; Duchefa). Plates were kept for 4d at 4ºC in dark. To induce germination, plates were transferred to 200 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) light for 4 h and, subsequently, wrapped in aluminum-foil. Thereafter, the plates were left in darkness for 5 days at 20ºC. Seedlings were photographed and hypocotyl length was measured using ImageJ software (Abramoff et al., 2004).

Ethylene release measurements were performed on 30 day-old plants, 1.5 ± 1 h after start of the photoperiod, as described in (Millenaar et al., 2005, 2009). Whole rosettes of about 300 mg were weighed and then placed in a syringe with a volume of 1.5 ml. Ethylene was allowed to accumulate in the syringe for 15-20 min. Subsequently, the air was analyzed on a gas chromatograph (GC955, Synspec). This short time frame prevented wound-induced ethylene production, which started to accumulate only after 20 min.

**Mathematical analysis of cell proliferation rates**

The mathematical model to predict cell proliferation rates is similar to the one described in Polko et al. (2012a). The background on secondary measurements of cell proliferation is explained in Supplemental Text S1, parameters are in Supplemental Table S3. Petiole shape was quantified by fitting a function through the measured petiole angle data describing the ‘proximal’ angle (petiole emergence from the shoot) and the ‘distal’ angle (intersection petiole and leaf blade). A smooth function was fitted to the measured cell lengths along the petiole to correct for variability. Given that along the adaxial side hardly significant differences were found in cell lengths (Fig. 4A), the adaxial cell length data were fitted collectively to a single overarching function. In contrast, the abaxial cell length data showed significant differences in the proximal part (Fig. 4B) and were fitted for each individual dataset independently. Since no significant differences in cell length were found in the distal part of the petiole, an extra constraint was added which required that the maximum cell length (in the distal part of the petiole) would be the same for the different datasets (Supplemental Table S3).

The curve describing petiole shape at \(t=0\) h was divided into 50 sections of 200 \(\mu\)m. To each section an arc was fitted, and by combining the curve of the arc with the function fitted to the measured cell lengths, the number of ad- and abaxial cells per section could be calculated (See Supplemental Text S2 and Supplemental Table S3). The cell number per section at \(t=0\) h, combined with the functions describing the ad- and abaxial cell lengths for the 10 h control and ethylene treatments allowed calculation of predicted petiole shape after 10 h treatment for the null hypothesis, which assumes no cell proliferation during the treatments. Deviation from the predicted petiole shape to the observed shape allowed prediction of adaxial or abaxial cell proliferation (Supplemental Text S3). Since overall petiole elongation was not taken into account, the obtained cell proliferation rates represent relative rather than absolute values.

**In silico model of hyponastic growth**
In the *in silico* model, the (hyponastic) growth of the petiole was simulated for ten hours using one-hour time intervals. During each time step, cells expanded and/or divided. Cells could only divide after reaching a specified minimal length, after which there was a probability to divide, evaluated at each time step. Simulations were initiated with a petiole that consisted of cells that were randomly selected from a population of *in silico* growing and dividing cells. This population was generated by simulating cell elongation and cell division for 10,000 time steps, starting from a single cell. All simulations were repeated 1,000 times (for parameters of all simulations see Supplemental Table S4). For the results shown in Fig. 5D-G, the initial (abaxial and adaxial) length of the petiole was set such that the shape of the petiole resembles observations for wild-type at t=0 h. Supplemental Text S5 discusses the relation between cell length and elongation for linear, exponential, logistic and exponential growth, as well as the calculation of the petiole curvature (for results shown in Fig. 5B,C) and differential (hyponastic) growth (for results shown in Fig. 5D-G).

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Literature cited

1. Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics International 11: 36-42

2. Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira, A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell 16: 2463-2480

3. Beemster GT, Baskin TI (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. Plant Physiol 116: 1515-1526

4. Benschop JJ, Millenaar FF, Smeets, ME, van Zanten M, Voesenek LACJ, Peeters AJM (2007) Abscisic acid antagonizes ethylene-induced hyponastic growth in Arabidopsis. Plant Physiol 143: 1013-1023

5. Bridge LJ, Franklin KA, Homer ME (2013) Impact of plant shoot architecture on leaf cooling: a coupled heat and mass transfer model. J Roy Soc Interface 10: 20130326

6. Boudolf V, Lammens T, Boruc J, Van Leene J, Maes S, van Isterdael G, Russinova E, Kondorosi E, Witters E, De Jaeger G, Inzé, D, De Veylder L (2009) CDKB1;1 forms a functional complex with CYCA2;3 to suppress endocycle onset. Plant Physiol 150: 1482-1493

7. Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Gorlach J (2001) Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. Plant Cell 13: 1499-1510

8. Burssens S, Engler JD, Beeckman T, Richard C, Shaul O, Ferreira P, Van Montagu M, Inze D (2000) Developmental expression of the Arabidopsis thaliana CycA2;1 gene. Planta 211: 623-631

9. Cheniclet C, Rong W, Causse M, Frangne N, Bolling L, Carde J, Renaudin J (2005) Cell expansion and endoreduplication show a large genetic variability in pericarp and contribute strongly to tomato fruit growth. Plant Physiol 139: 1984-1994

10. Colón-Carmona, A, You R, Haimovitch-Gal T, Doerner P (1999) Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J 20: 503-508.

11. Cox MC, Millenaar FF, Van Berkel YE, Peeters AJ, Voesenek LA (2003) Plant movement. Submergence-induced petiole elongation in Rumex palustris depends on hyponastic growth. Plant Physiol 132: 282-291

12. Dewitte W, Murray JAH (2003) The plant cell cycle. Annu Rev Plant Biol 54: 235-264.
17. Dornbusch T, Michaud O, Xenarios I, Fankhauser C (2014) Differentially phased leaf growth and movements in Arabidopsis depend on coordinated circadian and light regulation Plant Cell 26: 3911-3921
18. Etchells JP, Provost CM, Turner SR (2012) Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. PLoS Genet 8: e1002997
19. Gendreau E, Orbovic V, Hofte H, Traas J (1999) Gibberellin and ethylene control endoreduplication levels in the Arabidopsis thaliana hypocotyl. Planta 209: 513-516
20. Grieneisen VA, Xu J, Marée AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature 449: 1008-1013
21. Guzman P, Ecker JR (1990) Exploiting the triple response of Arabidopsis to identify ethylene-related mutants. Plant Cell 2: 513-523
22. Harashima H, Schnittger A (2010) The integration of cell division, growth and differentiation. Curr Opin Plant Biol 13: 66-74
23. Himmelspach R, Nick P (2001) Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212: 184-189
24. Imai KK, Ohashi Y, Tsuge T, Yoshizumi T, Matsui M, Oka A, Aoyama T (2006) The A-type cyclin CYCA2;3 is a key regulator of ploidy levels in Arabidopsis endoreduplication. Plant Cell 18: 382-396
25. Inze D, De Veylder L (2006) Cell cycle regulation in plant development. Annu Rev Genet 40: 77-105
26. Karimi M, Inze D, Depicker A (2002) GATEWAY((TM)) vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci 7: 193-195
27. Keuskamp DH, Pollmann S, Voesenek LACJ, Peeters AJM, Pierik R (2010) Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. Proc Natl Acad Sci USA 107: 22740-22744
28. Keller MM, Jaillais Y, Pedmale UV, Moreno JE, Chory J, Ballaré CL (2011) Cryptochrome 1 and phytochrome B control shade avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant J 67: 195-207
29. Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2009) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr. Biol 19: 408-413
30. Kozuka T, Horiguchi G, Kim GT, Ohgishi M, Sakai T, Tsukaya H (2005) The different growth responses of the Arabidopsis thaliana leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. Plant Cell Physiol 46: 213-223
31. Kudo N, Kimura Y (2002) Nuclear DNA endoreduplication during petal development in cabbage: Relationship between ploidy levels and cell size. J Exp Bot 53: 1017-1023
32. Lorbiecke R, Sauter M (1999) Adventitious root growth and cell-cycle induction in deepwater rice Plant Physiol 119: 21-30
33. Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B (2009) Ethylene is an endogenous stimulator of cell division in the cambial meristem of Populus. Proc Natl Acad Sci U S A 106: 5984-5989
34. Liu YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8: 457-463
Mariconti L, Pellegrini B, Cantoni R, Stevens R, Bergounioux C, Cella R, Albani D (2002) The E2F family of transcription factors from Arabidopsis thaliana - novel and conserved components of the retinoblastoma/E2F pathway in plants. J Biol Chem 277: 9911-9919

Millenaar FF, Cox MC, van Berkel YE, Welschen RA, Pierik R, Voesenek LACJ, Peeters AJM (2005) Ethylene-induced differential growth of petioles in Arabidopsis. Analyzing natural variation, response kinetics, and regulation. Plant Physiol 137: 998-1008

Millenaar FF, van Zanten M, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2009) Differential petiole growth in Arabidopsis thaliana: Photocontrol and hormonal regulation. New Phytol 184: 141-152

Moreno JE, Tao Y, Chory J, Ballaré CL (2009) Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc Natl Acad Sci USA 106: 4935-4940

Mullen JL, Weing C, Hangarter RP (2006) Shade avoidance and the regulation of leaf inclination in Arabidopsis. Plant Cell Environ 29: 1099-1106

Nakano T, Suzuki K, Ohtsuki N, Tsujimoto Y, Fujimura T, 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

Ortega-Martínez O, Pernas M, Carol RJ, Dolan L (2007) Ethylene modulates stem cell division in the Arabidopsis thaliana root. Science 317: 507-510

Peña-Castro J, Van Zanten M, Lee SC, Patel M, Voesenek LACJ, Fukao T, Bailey-Serres J (2011) Expression of rice SUB1A and SUB1C transcription factors in Arabidopsis uncovers flowering inhibition as a submergence-tolerance mechanism. Plant J 67: 434-446

Pierik R, Tholen D, Poorter H, Visser EJ, Voesenek LA (2006) The Janus face of ethylene: Growth inhibition and stimulation. Trends Plant Sci 11: 176-183

Pillitteri LJ, Bemis SM, Shpak ED, Torri KU (2007) Haploinsufficiency after successive loss of signaling reveals a role for ERECTA-family genes in Arabidopsis ovule development. Development 134: 3099-3109

Polko JK, van Zanten M, van Rooij, JA, Marée AFM, Voesenek LACJ, Peeters AJM, Pierik R (2012a) Ethylene-induced differential petiole growth in Arabidopsis thaliana involves local microtubule reorientation and cell expansion. New Phytol 193: 339-348

Polko JK, Temanni MR, van Zanten M, van Workum W, Iburg S, Pierik R, Voesenek LACJ, Peeters AJM (2012b) Illumina sequencing technology as a method of identifying T-DNA insertion loci in activation-tagged Arabidopsis thaliana plants. Mol Plant 5: 948-950

Polko JK, Pierik R, Zanten M, Tarkowská D, Strnad M, Voesenek LACJ, Peeters AJM (2013) Ethylene promotes hyponastic growth through interaction with ROTUNDIFOLIA3/CYP90C1 in Arabidopsis. J Exp Bot 74: 6213-623

Polyn S, Willems A, De Veylder L (2015) Cell cycle entry, maintenance, and exit during plant development. Curr Opin Plant Biol 23: 1-7

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two arabidopsis F box proteins: EBF1 and EBF2. Cell. 115: 679-689.
8140. Rauf M, Arif M, Fisahn J, Xue G-P, Balazadeh S, Mueller-Roeber B (2013) NAC Transcription Factor SPEEDY HYPONASTIC GROWTH Regulates Flooding-Induced Leaf Movement in Arabidopsis. Plant Cell 25: 4941–4955

8141. Richard C, Granier C, Inze D, De Veylder L (2001) Analysis of cell division parameters and cell cycle gene expression during the cultivation of Arabidopsis thaliana cell suspensions. J Exp Bot 52: 1625-1633

8142. Roeder AHK, Chickarmane V, Cunha A, Obara B, Manjunath BS, Meyerowitz EM (2010) Variability in the control of cell division underlies sepal epidermal patterning in Arabidopsis thaliana. PLoS Biol 8: e1000367

8143. Savaldi-Goldstein S, Petro C, Chory J (2007) The epidermis both drives and restricts plant shoot growth. Nature 446: 1990-202

8144. Skirycz A, Claeyys H, De Bodt S, Oikawa A, Shinoda S, Andriankaja M, Maleux K, Eloy NB, Coppens F, Yoo SD, Saito K, Inzé D (2011) Pause-and-stop: the effects of osmotic stress on cell proliferation during early leaf development in Arabidopsis and a role for ethylene signaling in cell cycle arrest. Plant Cell 23: 1876-1888

8145. Sugimoto-Shirasu K, Roberts K (2003) "Big it up": Endoreduplication and cell-size control in plants. Curr Opin Plant Biol 6: 544-553.

8146. Ueda K, Matsuyama T, Hashimoto T (1999) Visualization of microtubules in living cells of transgenic Arabidopsis thaliana. Protoplasma 206: 201-206

8147. Van Zanten M, Voesenek LACJ, Peeters AJM, Millenaar FF (2009) Hormone- and light-mediated regulation of heat-induced differential petiole growth in Arabidopsis. Plant Physiol 151: 1446-1458

8148. Van Zanten M, Pons TL, Janssen JAM, Voesenek LACJ, Peeters AJM (2010a) On the relevance and control of leaf angle. Crit Rev Plant Sci 29: 300-316

8149. Van Zanten M, Snoek LB, van Eck-Stouten E, Proveniers MCG, Torii KU, Voesenek LACJ, Peeters AJM, Millenaar FF (2010b) Ethylene-induced hyponastic growth in Arabidopsis thaliana is controlled by ERECTA. Plant J 61: 83-95

8150. Vandepoele K, Raes J, De Veylder L, Rouze P, Rombauts S, Inzé D (2002) Genome-wide analysis of core cell cycle genes in Arabidopsis. Plant Cell 14: 903-916

8151. Vanneste S, Coppens F, Lee E, Donner TJ, Xie Z, Van Isterdael G, Dhondt S, De Winter F, De Rybel B, Vuylsteke M, De Veylder L, Friml J, Inzé D, Grotewold E, Scarpella E, Sack F, Beemster GT, Beeckman T (2011) Developmental regulation of CYCA2s contributes to tissue-specific proliferation in Arabidopsis. EMBO J 30: 3430-3441

8152. Vasseur F, Pantin F, Vile D (2011) Changes in light intensity reveal a major role for carbon balance in Arabidopsis responses to high temperature. Plant Cell Environ 34: 1563-1576

8153. Weigel D, Ahn JH, Blázquez MA, Borevitz JO, Christensen SK, Fankhauser C, Ferrándiz C, Kardailsky I, Malancharuvil EJ, Neff MM, Nguyen JT, Sato S, Wang ZY, Xia Y, Dixon RA, Harrison MJ, Lamb CJ, Yanofsky MF, Chory J (2000) Activation tagging in Arabidopsis. Plant Physiol 122: 1003-1013

8154. Weiste C, Iven T, Fischer U, Onate-Sanchez L, Droege-Laser W (2007) In planta ORFeome analysis by large-scale over-expression of GATEWAY (R)-compatible cDNA clones: Screening of ERF transcription factors involved in abiotic stress defense. Plant J 52: 382-390
855. **Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Blilou I, Korthout H, Chatterjee J, Mariconti L, Gruissem W, Scheres B** (2005) The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in Arabidopsis roots. Cell. **123**: 1337-13349

856. **Yoshizumi T, Tsumoto Y, Takiguchi T, Nagata N, Yamamoto YY, Kawashima M, Ichikawa T, Nakazawa M, Yamamoto N, Matsui M** (2006) Increased level of polyploidy1, a conserved repressor of CYCLINA2 transcription, controls endoreduplication in Arabidopsis. Plant Cell **18**: 2452-2468

857. **Yu Y, Steinmetz A, Meyer D, Brown S, Shen WH** (2003) The tobacco A-type cyclin, nicta;CYCA3;2, at the nexus of cell division and differentiation. Plant Cell **15**: 2763-2777
Figure legends

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for details. A, Graphical representation of the model at different time-points during a simulation of a
wild-type Col petiole. The abaxial and the adaxial cell layers are each represented by a single
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Table I. Relative expression levels (± SEM) of genes flanking the *EHY-D* T-DNA insertion, compared to the wild-type Col, in control (air 3 h) and ethylene-enriched conditions (3 h) (see also Fig. 1G).

| AGI-code     | Gene ontology (GO annotation)                        | Relative Expression ± SEM |
|--------------|------------------------------------------------------|---------------------------|
| At5g25430    | HCO3-Anion exchange protein family protein          | Col 1.00 ± 0.16 Ethylene 1.17 ± 0.15 Col 1.15 ± 0.18 Ethylene 0.84 ± 0.10 |
| At5g25420    | Xanthine/uracil/vitamin C permease                  | Col 1.00 ± 0.30 Ethylene 0.77 ± 0.62 Col 0.62 ± 0.48 Ethylene 1.13 ± 0.39 |
| At5g25415    | Unknown protein                                     | Col 1.00 ± 0.60 Ethylene 1.91 ± 1.40 Col 2.84 ± 1.58 Ethylene 0.50 ± 0.26 |
| At5g25410    | Unknown protein                                     | Col 1.00 ± 0.31 Ethylene 0.65 ± 0.26 Col 2.56 ± 1.47 Ethylene 0.90 ± 0.69 |
| At5g25400    | Nucleotide-sugar transporter family protein         | Col 1.00 ± 0.19 Ethylene 1.22 ± 0.26 Col 1.48 ± 0.49 Ethylene 1.01 ± 0.14 |
| At5g25390    | ERF/AP2 B-6 transcription factor, SHN3              | Col 1.00 ± 0.28 Ethylene 3.18 ± 0.95 Col 1.11 ± 0.53 Ethylene 3.24 ± 0.43 |
| At5g25380    | CYCA2;1, core cell cycle regulator                  | Col 1.00 ± 0.30 Ethylene 142.45 ± 21.71 Col 0.89 ± 0.30 Ethylene 106.58 ± 13.61 |
| At5g25370    | phospholipase-D α3                                  | Col 1.00 ± 0.15 Ethylene 1.13 ± 0.13 Col 1.13 ± 0.23 Ethylene 1.19 ± 0.24 |
| At5g25360    | Unknown protein                                     | Col 1.00 ± 0.06 Ethylene 2.24 ± 0.48 Col 2.23 ± 0.21 Ethylene 1.12 ± 0.08 |
| At5g25350    | EIN3-binding F-box protein 2 (EBF2)                 | Col 1.00 ± 0.12 Ethylene 2.27 ± 0.61 Col 2.92 ± 0.96 Ethylene 0.66 ± 0.19 |
**Supplemental Files**

- **Figure S1.** Hyponastic response of plants ectopically expressing transcription factor *SHN3 ERF/AP2 B-6*.
- **Figure S2.** qRT-PCR analysis of *CYCA2;1* expression in independent *35S::CYCA2;1* transformants and *EHY-D*.
- **Figure S3.** Correlation analysis between *CYCA2;1* expression and amplitude of hyponastic growth in response to ethylene.
- **Figure S4.** Hyponastic response of *cyca2;1* mutants upon low light and high temperature treatment and expression of *A2-type CYCLINS*.
- **Figure S5.** Hyponastic growth response of *35S:CYCA2;2-1* and *A2-type CYCLIN double- and triple mutant combination*.
- **Figure S6.** Histochemical analysis of *A2-Cyclin* promoter activity in rosettes and petioles.
- **Figure S7.** Histochemical analysis of ethylene effects on *A2-Cyclin* promoter activity in petioles
- **Figure S8.** Effects of ethylene on endoreduplication in petioles.
- **Figure S9.** Ethylene-induced CMT reorientation in *35S::CYCA2;1* does not differ from wild-type.
- **Figure S10.** Ethylene treatment prior to low light treatment does not affect kinetics of low light-induced hyponastic growth.

- **Material and Methods S1**

- **Text S1.** Indirect measurements of cell division and expansion rates.
- **Text S2.** Deriving the number of cells from petiole shape and cell lengths.
- **Text S3.** Profiling of cell proliferation along the petiole.
- **Text S4.** Influence of cell division on tissue growth.
- **Text S5.** Additional information for the *in silico* model of petiole.

- **Table S1.** Initial petiole angles at t=0h, of *A2-type CYCLIN*-related mutants described in this work.
- **Table S2.** Primers used for Real-time qRT-PCR.
- **Table S3.** Parameters used to profile cell proliferation rates.
- **Table S4.** Parameters used in the *in silico* model.

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Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophotonics International 11: 36-42

Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira, A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell 16: 2463-2480

Beemster GT, Baskin TI (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. Plant Physiol 116: 1515-1526

Benschop JJ, Millenaar FF, Smeeets, ME, van Zanten M, Voesenek LA CJ, Peeters A JM (2007) Abscisic acid antagonizes ethylene-induced hyponastic growth in Arabidopsis. Plant Physiol 143: 1013-1023

Bridge LJ, Franklin KA, Homer ME (2013) Impact of plant shoot architecture on leaf cooling: a coupled heat and mass transfer model. J Roy Soc Interface 10: 20130326

Boudolf V, Lammens T, Boruc J, Van Leene J, van Den Daele H, Maes S, van Isterdael G, Russinova E, Kondorosi E, Dejaeger G, Inze D, De Veylder L (2009) CDKB1;1 forms a functional complex with CYCA2;3 to suppress endocycle onset. Plant Physiol 150: 1482-1493

Boyes DC, Zayed AM, Ascenzi R, McCaskill AJ, Hoffman NE, Davis KR, Gorlach J (2001) Growth stage-based phenotypic analysis of Arabidopsis: A model for high throughput functional genomics in plants. Plant Cell 13: 1499-1510

Bursssens S, Engler JD, Beeckman T, Richard C, Shaui O, Ferreira P, Van Montagu M, Inze D (2000) Developmental expression of the Arabidopsis thaliana CycA2;1 gene. Planta 211: 623-631

Cheniclet C, Rong W, Causse M, Frangne N, Bolling L, Carde J, Renaudin J (2005) Cell expansion and endoreduplication show a large genetic variability in pericarp and contribute strongly to tomato fruit growth. Plant Physiol 139: 1984-1994

Colón-Carmona, A, You R, Haimovitch-Gal T, Doerner P (1999) Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J 20: 503-508.

Cox MC, Millenaar FF, Van Berkel YE, Peeters AJ, Voesenek LA (2003) Plant movement. Submergence-induced petirole elongation in Rumex palustris depends on hyponastic growth. Plant Physiol 132: 282-291

Cox MCH, Benschop JJ, Vreeburg RM, Wagemakers CAM, Moritz T, Peeters AJM, Voesenek LACJ (2004) The roles of ethylene, auxin, abscisic acid, and gibberellin in the hyponastic growth of submerged Rumex palustris petioles. Plant Physiol 136: 2948-2960

Crawford AJ, McLachlan D, Hetherington AM, Franklin KA (2012) High Temperature exposure increases plant cooling capacity. Curr Biol 22: R396-R397
Dan H, Imaeke H, Wasteneys G, Kazama H (2003) Ethylene stimulates endoreduplication but inhibits cytokinesis in cucumber hypocotyl epidermis. Plant Physiol 133: 1726-1731

De Wit M, Kege W, Evers JB, Vergeer-van Eijk MH, Gankema P, Voesenek LACJ, Pierik R (2012) Plant neighbor detection through touching leaf tips precedes phytochrome signals. Proc Natl Acad Sci USA 109: 14705-14710

Dewitte W, Murray JAH (2003) The plant cell cycle. Annu Rev Plant Biol 54: 235-264.

Dornbusch T, Michaud O, Xenarios I, Fankhauser C (2014) Differentially phased leaf growth and movements in Arabidopsis depend on coordinated circadian and light regulation Plant Cell 26: 3911-3921

Etchells JP, Provost CM, Turner SR (2012) Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. PLoS Genet 8: e1002997

Gendreau E, Orbovic V, Hoft H, Traas J (1999) Gibberellin and ethylene control endoreduplication levels in the Arabidopsis thaliana hypocotyl. Planta 209: 513-516

Grieneisen VA, Xu J, Marée AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. Nature 449: 1008-1013

Guzman P, Ecker JR (1990) Exploiting the triple response of Arabidopsis to identify ethylene-related mutants. Plant Cell 2: 513-523

Harashima H, Schnittger A (2010) The integration of cell division, growth and differentiation. Curr Opin Plant Biol 13: 66-74

Himmelspach R, Nick P (2001) Gravitropic microtubule reorientation can be uncoupled from growth. Planta 212: 184-189

Imai KK, Ohashi Y, Tsuge T, Yoshizumi T, Matsui M, Oka A, Aoyama T (2006) The A-type cyclin CYCA2;3 is a key regulator of ploidy levels in Arabidopsis endoreduplication. Plant Cell 18: 382-396

Inze D, De Veylder L (2006) Cell cycle regulation in plant development. Annu Rev Genet 40: 77-105

Karimi M, Inze D, Depicker A (2002) GATEWAY((TM)) vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci 7: 193-195

Keuskamp DH, Pollmann S, Voesenek LACJ, Peeters AJM, Pierik R (2010) Auxin transport through PIN-FORMED 3 (PIN3) controls shade avoidance and fitness during competition. Proc Natl Acad Sci USA 107: 22740-22744
Keller MM, Jallais Y, Pedmale UV, Moreno JE, Chory J, Ballaré CL (2011) Cryptochrome 1 and phytochrome B control shade avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant J 67: 195-207

Koizumi MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2009) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr. Biol 19: 408-413

Kozuka T, Horiguchi G, Kim GT, Ohgishi M, Sakai T, Tsukaya H (2005) The different growth responses of the Arabidopsis thaliana leaf blade and the petiole during shade avoidance are regulated by photoreceptors and sugar. Plant Cell Physiol 46: 213-223

Kudo N, Kimura Y (2002) Nuclear DNA endoreduplication during petal development in cabbage: Relationship between ploidy levels and cell size. J Exp Bot 53: 1017-1023

Lorbiecke R, Sauter M (1999) Adventitious root growth and cell-cycle induction in deepwater rice Plant Physiol 119: 21-30

Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B (2009) Ethylene is an endogenous stimulator of cell division in the cambial meristem of Populus. Proc Natl Acad Sci USA 106: 5984-59

Liu YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8: 457-463

Mariconti L, Pellegrini B, Cantoni R, Stevens R, Bergounioux C, Cella R, Albani D (2002) The E2F family of transcription factors from Arabidopsis thaliana - novel and conserved components of the retinoblastoma/E2F pathway in plants. J Biol Chem 277: 9911-9919

Millenaar FF, Cox MC, van Berkel YE, Welschen RA, Pierik R, Voesenek LACJ, Peeters AJM (2005) Ethylene-induced differential growth of petioles in Arabidopsis. Analyzing natural variation, response kinetics, and regulation. Plant Physiol 137: 998-1008

Millenaar FF, van Zanten M, Cox MCH, Pierik R, Voesenek LACJ, Peeters AJM (2009) Differential petiole growth in Arabidopsis thaliana: Photocontrol and hormonal regulation. New Phytol 184: 141-152

Moreno JE, Tao Y, Chory J, Ballaré CL (2009) Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc Natl Acad Sci USA 106: 4935-4940

Mullen JL, Weinig C, Hangarter RP (2006) Shade avoidance and the regulation of leaf inclination in Arabidopsis. Plant Cell Environ 29: 1099-1106

Nakano T, Suzuki K, Ohtsuki N, Tsujimoto Y, Fujimura T, 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

Ortega-Martinez O, Pernas M, Carol RJ, Dolan L (2007) Ethylene modulates stem cell division in the Arabidopsis thaliana root. Science 317: 507-510
Peña-Castro J, Van Zanten M, Lee SC, Patel M, Voesenek LACJ, Fukao T, Bailey-Serres J (2011) Expression of rice SUB1A and SUB1C transcription factors in Arabidopsis uncovers flowering inhibition as a submersion-tolerance mechanism. Plant J 67: 434-446

Pierik R, Tholen D, Poorter H, Visser EJ, Voesenek LA (2006) The Janus face of ethylene: Growth inhibition and stimulation. Trends Plant Sci 11: 176-183

Pillitteri LJ, Bemis SM, Shpak ED, Torii KU (2007) Haploinsufficiency after successive loss of signaling reveals a role for ERECTA-family genes in Arabidopsis ovule development. Development 134: 3099-3109

Polko JK, van Zanten M, van Rooij, JA, Marée AFM, Voesenek LACJ, Peeters AJM, Pierik R (2012a) Ethylene-induced differential petiole growth in Arabidopsis thaliana involves local microtubule reorientation and cell expansion. New Phytol 193: 339-348

Polko JK, Temanni MR, van Zanten M, van Workum W, Ibarg S, Pierik R, Voesenek LACJ, Peeters AJM (2012b) Illumina sequencing technology as a method of identifying T-DNA insertion loci in activation-tagged Arabidopsis thaliana plants. Mol Plant 5: 948-950

Polko JK, Pierik R, Zanten M, Tarkowská D, Strnad M, Voesenek LA CJ, Peeters A JM (2013) Ethylene promotes hyponastic growth through interaction with ROTUNDIFOLIA3/CYP90C1 in Arabidopsis. J Exp Bot 74: 6213-623

Polyn S, Willems A, De Veylder L (2015) Cell cycle entry, maintenance, and exit during plant development. Curr Opin Plant Biol 23: 1-7

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two arabidopsis F box proteins: EBF1 and EBF2. Cell. 115: 679-689.

Rauf M, Arif M, Fisahn J, Xue G-P, Balazadeh S, Mueller-Roeber B (2013) NAC Transcription Factor SPEEDY HYPONASTIC GROWTH Regulates Flooding-Induced Leaf Movement in Arabidopsis. Plant Cell 25: 4941-4955

Richard C, Granier C, Inze D, De Veylder L (2001) Analysis of cell division parameters and cell cycle gene expression during the cultivation of Arabidopsis thaliana cell suspensions. J Exp Bot 52: 1625-1633

Roeder AHK, Chickarmane V, Cunha A, Obara B, Manjunath BS, Meyerowitz EM (2010) Variability in the control of cell division underlies sepal epidermal patterning in Arabidopsis thaliana. PLoS Biol 8: e1000367

Savaldi-Goldstein S, Petro C, Chory J (2007) The epidermis both drives and restricts plant shoot growth. Nature 446: 1990-202

Skiyrzyca A, Claeyes H, De Bodt S, Oikawa A, Shinoda S, Andriankaja M, Maleux K, Eloy NB, Coppens F, Yoo SD, Saito K, Inzé D (2011) Pause-and-stop: the effects of osmotic stress on cell proliferation during early leaf development in Arabidopsis and a role for ethylene signaling in cell cycle arrest. Plant Cell 23: 1876-1888
Sugimoto-Shirasu K, Roberts K (2003) "Big it up": Endoreduplication and cell-size control in plants. Curr Opin Plant Biol 6: 544-553.

Ueda K, Matsuym a T, Hashimoto T (1999) Visualization of microtubules in living cells of transgenic Arabidopsis thaliana. Protoplasma 206: 201-206

Van Zanten M, Voesenek LACJ, Peeters AJM, Millenaar FF (2009) Hormone- and light-mediated regulation of heat-induced differential petiole growth in Arabidopsis. Plant Physiol 151: 1446-1458

Van Zanten M, Pons TL, Janssen JAM, Voesenek LACJ, Peeters AJM (2010a) On the relevance and control of leaf angle. Crit Rev Plant Sci 29: 300-316

Van Zanten M, Snoek LB, van Eck-Stouten E, Proveniers MCG, Torii KU, Voesenek LACJ, Peeters AJM, Millenaar FF (2010b) Ethylene-induced hyponastic growth in Arabidopsis thaliana is controlled by ERECTA. Plant J 61: 83-95

Vandepoele K, Raes J, De Veylder L, Rouze P, Rombouts S, Inzé D (2002) Genome-wide analysis of core cell cycle genes in Arabidopsis. Plant Cell 14: 903-916

Vanneste S, Coppens F, Lee E, Donner TJ, Xie Z, Van Isterdael G, Dhondt S, De Wintere F, De Rybel B, Vuylske te M, De Veylder L, Friml J, Inzé D, Grotewold E, Scarpella E, Sak F, Beerster GT, Beeckman T (2011) Developmental regulation of CYCA2s contributes to tissue-specific proliferation in Arabidopsis. EMBO J 30: 3430-3441

Van Zanten M, Onate-Sanchez L, Droege-Laser W (2007) In planta ORFeome analysis by large-scale over-expression of GATEWAY (R)-compatible cDNA clones: Screening of ERF transcription factors involved in abiotic stress defense. Plant J 52: 382-390

Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Billou I, Korthout H, Chatterjee J, Mariconti L, Gruissem W, Scheres B (2005) The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in Arabidopsis roots. Cell. 123: 1337-13349

Yoshizumi T, Tsumoto Y, Takiguchi T, Nagata N, Yamamoto YY, Kawashima M, Ichikawa T, Nakazawa M, Yamamoto N, Matsui M (2006) Increased level of polyploidy1, a conserved repressor of CYCLINA2 transcription, controls endoreduplication in Arabidopsis. Plant Cell 18: 2452-2468

Yu Y, Steinmetz A, Meyer D, Brown S, Shen WH (2003) The tobacco A-type cyclin, nicta;CYCA3;2, at the nexus of cell division and differentiation. Plant Cell 15: 2763-2777
