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Role of cytokinin and salicylic acid in plant growth at low temperatures

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Abstract Low temperature restrains plant growth by inhibiting the cell cycle, and phytohormones play important roles in this case; however, the molecular mechanisms whereby phytohormones affect growth at low temperature are largely unknown. When grown at 23, 16, 10, and 4°C, we found that *Arabidopsis thaliana* could develop with normal morphology, but needed a prolonged period of cultivation. By screening mutants, we could implicate cytokinin and salicylic acid. At 4°C, both *amp1* plants, which have an increased level of cytokinin, and wild-type plants treated with exogenous cytokinin, displayed relative growth rates greater than control by increasing total cell number. Additionally, transgenic *NahG* plants, which have lower salicylic acid content, grew faster than wild-type accompanied by larger cells. Expression of C-repeat binding transcription factors (CBFs), that mediate cold acclimation by stimulation of the expression of cold-inducible genes, was similar in all tested genotypes. Thus CBF expression did not correlate with the observed enhanced growth in mutants. The improved growth coincided with elevated expression of *CYCD3;1*, especially in *NahG* plants. At 4°C, enhanced endoreduplication took responsibility for larger cells in *NahG* plants, while enhanced cell division was observed in *amp1* plants.

Keywords *Arabidopsis thaliana* · Cell cycle · Low temperature · *amp1* · *NahG*

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Introduction

Plants are frequently subjected to low temperature, one of the most important environmental factors that greatly influence growth, development, survival, crop productivity, and species distribution (Lyons 1973; Levitt 1980). Many plants can acquire tolerance in response to low temperatures, which is known as cold acclimation (Thomashow 1999), including cool acclimation and freezing acclimation. Chilling (0–15°C) or freezing (<0°C) temperatures can cause injury and reduce growth depending on the cold tolerance of the species (Schneider et al. 1995; Pearce 1999).

Many plants of temperate origin, including *Arabidopsis thaliana*, are cold-tolerant and can grow to maturity at chilling temperatures. Cold acclimation induces the expression of the C-repeat binding transcription factors (CBFs), which in turn activate

many downstream genes, including *COR* (cold regulated), *KIN* (cold induced), *LTI* (low temperature induced), and *RD* (responsive to desiccation) genes (Kurkela and Borg-Franck 1992; Gilmour et al. 1992, 1998; Nordin et al. 1993; Yamaguchi-Shinozaki and Shinozaki 1994; Zhu et al. 2007). Although plants acquire a tolerance during cold acclimation to survive at low temperature, some important agricultural characters, such as whole plant size, morphogenesis, and reproduction need to be better understood in responses to low temperature, particularly in terms of molecular regulation.

Plant growth results from cell division and expansion. Low temperature decreases plant growth rate and prolongs the cell cycle. The cell cycle duration in the root meristem of *Zea mays* increased 21-fold as the temperature was decreased from 25 to 3°C (Francis and Barlow 1988). In the root meristem of *Allium cepa*, when temperature was decreased from 25 to 10°C, cell cycle duration increased from 17.6 to 69 h (Giménez-Abián et al. 2004). An analysis of maize leaf growth showed that chilling temperature increased the cell cycle extent by 64% compared to normal temperature, but in contrast, did not affect the size of mature cells, suggesting that the decreased growth rate at low temperature resulted from a prolonged span of cell cycle rather than a reduction in total cellular expansion (Rymen et al. 2007). However, the mechanisms associated with cell cycle regulation at low temperature remain unclear, although an investigation of the expression of genes related to the cell cycle was begun (Rymen et al. 2007).

Phytohormones and salicylic acid might have important roles for the ability of plants to sustain growth and development at cold temperatures. Arabidopsis *NahG* plants, which express the bacterial salicylate hydroxylase and thereby contain reduced amounts of salicylic acid, grow faster than wild-type at 5°C because of a greater cell expansion rather than because of cell number increase (Scott et al. 2004). At low temperature, the content of cytokinin could be decreased because low temperature is known to stimulate the activity of cytokinin-oxidase (Veselova et al. 2005), and the content of abscisic acid increases to induce expression of cold acclimation related genes (Xiong et al. 2001). Those results bring up a clue that the low temperature could alter phytohormone levels, prolong cell cycle process, and trigger cold acclimation signaling pathways; however, it is unclear how the cross-talk in these signaling pathway proceeds.

In this article, through strategies of mutant scanning and treatment with external phytohormone under constant 4°C conditions, we found that plant growth was inhibited by a prolonged duration of cell cycle, which was regulated to some extent by the balance between cytokinin and salicylic acid.

Materials and methods

Plant material

All wild-type and mutant lines of *Arabidopsis thaliana* (L.) Heynh are in the Columbia-0 background. The *NahG* line was kindly provided by Scott Uknes (Cropsolution, Research Triangle Park, NC). The mutant plants with modulated phytohormones (auxin, cytokinin, ethylene, brassinosteroid or gibberellin) transport, signaling or metabolism (*pin1, pin2, pin3, pin7, tir1-1, aux1-7, axr2; cre1, ahk2, ahk3, amp1,* 35s: *ckx1;ein2-1, ein3-1; det2-1, bri1-5; ga1-3*) were provided by ABRC if not specially indicated. For aseptic growth, all seeds were surface sterilized and plated on half-strength Murashige-Skoog medium (Sigma–Aldrich, USA) supplemented with 1% (w/v) sucrose. For cytokinin treatment, zeatin (Sigma, Z0164, 5% cis isomer and 93% trans isomer, USA) was added to half-strength Murashige-Skoog medium on plates, at concentration 0.01, 0.05, 0.1 and 0.5 μM. The seeds were treated in darkness at 4°C for 5 days before sowed into plates or pots and put into a growth chamber (Percival Scientific Environmental Chamber, USA) at 23°C to grow for 1 week, with a long-day photoperiod (16 h light:8 h dark), a fluence rate of 80–100 μmol m^{-2} s^{-1}, then transferred to a indicated temperature, unless otherwise indicated. Plant locations within the chambers were changed ten times monthly.

Analysis of weight, cell size and chlorophyll content

Dry weight measurements were made of oven-dried biomass of whole shoots without roots. Cell size for abaxial epidermis and mesophyll was measured on fully expanded rosette leaves. Leaves were cleared

and mounted on a compound microscope (DMRE, Leica, Germany) and imaged with a digital video camera (SPOT RT, Diagnostic Instruments, Sterling Heights) interfaced to a computer running software from the same manufacturer (SPOT 4.6). To measure leaf area, seedlings were scanned with a scanner (Perfection 4990, Epson) and total leaf area per seedling was obtained with image-analysis software (WinRHIZO, Philippines). Relative growth rates (RGRs) were estimated as the regression line gradient of in W against t over a period of exponential growth spanning five harvests. To measure chlorophyll content, leaves were ground in liquid nitrogen, extracted with 80% (v/v) acetone, and chlorophyll measured from absorbance at 645 and 663 nm as: $(A_{663} \times 0.00802 + A_{645} \times 0.0202) \times 1.5$ and normalized by fresh weight. The average data were calculated from at least three independent experiments (30–50 plants per experiment).

Analysis of gene expression

About 10-days-old wild-type, *amp1* and *NahG* seedlings (cotyledons and two visible leaves) were harvested from plates at 23 or 4°C. We extracted total RNA from the seedlings by using Trizol isolation reagent, as described in the manufacture's instructions (Sigma–Aldrich, USA). First-strand cDNA was synthesized from DNase-treated RNA with reverse transcriptase (Takara, Dalian, China) and oligo $(dT)_{18}$. The exponential phase of amplification was determined through optimizing cycle numbers for each template using control cDNA to allow the maximal detection of difference in transcript numbers. The following primers were designed for gene-specific transcript amplification.

CYCD2;1 (At2g22490): fw-5′-TGTCTTTCGGC AATTCATAGGAT-3′ and rv-5′-CAATTCCGATG ATGGGTTCTTC-3′; *CYCD3;1* (At4g34160): fw-5′-CTTCTCCATTCGTTGTTTTGC-3′ and rv-5′-GAG GGTCAAAGGGATCAACTTGC-3′; *CBF1* (At4g 25490): fw-5′-CCTTATCCAGTTTCTTGAAACAG AG-3′ and rv-5′-CGAATATTAGTAACTCCAAAG CGAC-3′; *CBF2* (At4g25470): fw-5′-CCTTATCC AGTTTCTTGAAACAGAG-3′ and rv-5′-CACTCG TTTCTCAGTTTTACAAAC-3′; *CBF3* (At4g25480): fw-5′-CCTTATCCAGTTTCTTGAAACAGAG-3′ and rv-5′-GACCATGAGCATCCGTCGTCATATGAC-3′; *ACTIN* (At3g12110): fw-5′-GATTTGGCATCACAC

TTTCTACAATG-3′ and rv-5′-GTTCCACCACTGA GCACAATG-3′.

Flow cytometry

Samples of fully expanded 4–6th rosette leaves of plants, which grew in pots for 20 days at 23°C and then were transferred to chamber at 4°C for 3.5 months, were harvested. To release the nuclei, the leaves were chopped with a razor blade in 500 μl ice-cold chopping buffer (45 mM MgCl₂, 30 mM sodium citrate, 20 mM 3-(N-morpholino) propane-sulfonic acid pH 7 and 0.1% Triton X-100) (Galbraith et al. 1991). The supernatant was filtered over a 20 μm mesh, and the nuclei were stained with 1 μl of 4′,6-diamidino-2-phenylindole (DAPI) from a stock of 1 mg ml⁻¹. The nuclei were measured with a flow cytometer (DB FACSAria™, San Jose, USA) and analyzed with the manufacturer's software.

Results

Low temperature prolonged growth to maturity

Low temperature inhibited plant growth. Arabidopsis is cold-tolerant and known to be able to grow to maturity at low temperature; however, it is not known to what extent growth to maturity in the cold alters morphological or reproductive phenotypes. Therefore, we grew plants for 15 days at 23°C and transferred them to chambers at 4, 10, 16 or 23°C until seed set was complete. The same long-day photoperiod was used throughout. We found that, as expected, growth decreased gradually with temperature, particularly at 4°C (Fig. 1a). Plants grew to maturity at all temperatures, but as temperature decreased, the time to flowering increased, being almost five times longer at 4°C than at 23°C (Fig. 1c). Moreover, temperature had almost no effect on morphology, or final plant size (Fig. 1b, d). Evidently, while low temperature suppressed the rate at which plants grow it did not appear to alter fundamental developmental processes.

Effect of low temperature on *amp1*

To identify molecular processes that link low temperature response and phytohormones, we screened

Fig. 1 Low temperature prolongs plant growth duration to ▶ maturation. **a** Plants at 23°C for 15 days and transferred them to chambers, respectively at 4, 10, 16 or 23°C for 20 days. **b** Normal phenotypes of bolting plants at 4, 10, 16 or 23°C, respectively for 120, 60, 40 and 30 days or so. **c** Bolting time of plants, respectively at 4, 10, 16 or 23°C. Bars are SE. **d** Maturity plants at 4 or 23°C, respectively for 150 or 50 days or so

dozens of phytohormone-related mutants for altered growth in the cold. We grew plants in plates for 1 week at 23°C and then transferred them to 4°C for additional 50 days. While nearly all the mutants behaved comparably or weakly to wild-type, an exception was *amp1*. This mutant grew slightly faster than wild-type at 23°C whereas at 4°C although growing about as slowly as the wild-type for the first 15 days of treatment, it accelerated after that so that the weight of *amp1* shoots by 50 days at 4°C reached almost that of 15-day plant grown at normal temperature, a time when the wild-type was about half its weight (Fig. 2a). These differences were confirmed quantitatively by assessing RGRs for accumulation of shoot dry mass. RGR over the first 18 days at 23°C was slightly, but significantly, higher in *amp1* whereas over the first 49 days at 4°C, RGR in *amp1* was nearly twice that of the wild-type (Fig. 2b). The phenotypic differences of *amp1* and wild-type plants after 1 month at 4°C were consistently observed in over ten experiments (Fig. 2e).

To assess the contributions of cell division and cell expansion to the growth of *amp1* plants at 4°C, adaxial epidermal cells and mesophyll cells were imaged on plants exposed to 4°C for 50 days, when it was obvious that the shoot weight differed between *amp1* and wild-type. We found that the size of both epidermal cells and mesophyll cells of *amp1* plants tended to be smaller than wild-type plants (Fig. 3a, b), and areas of rosette leaves of *amp1* plants were larger than wild-type (Fig. 3c); thus, continued cell division rather than enhanced expansion is associated with the better performance of *amp1* in the cold. To determine whether the difference in growth between the genotypes resulted from differences in the photosynthetic apparatus, we measured leaf chlorophyll content. No difference was detectable (Fig. 3d).

In addition to altered morphology, *amp1* contains elevated cytokinin levels (Chaudhury et al. 1993; Chin-Atkins et al. 1996; Nogué et al. 2000). To test
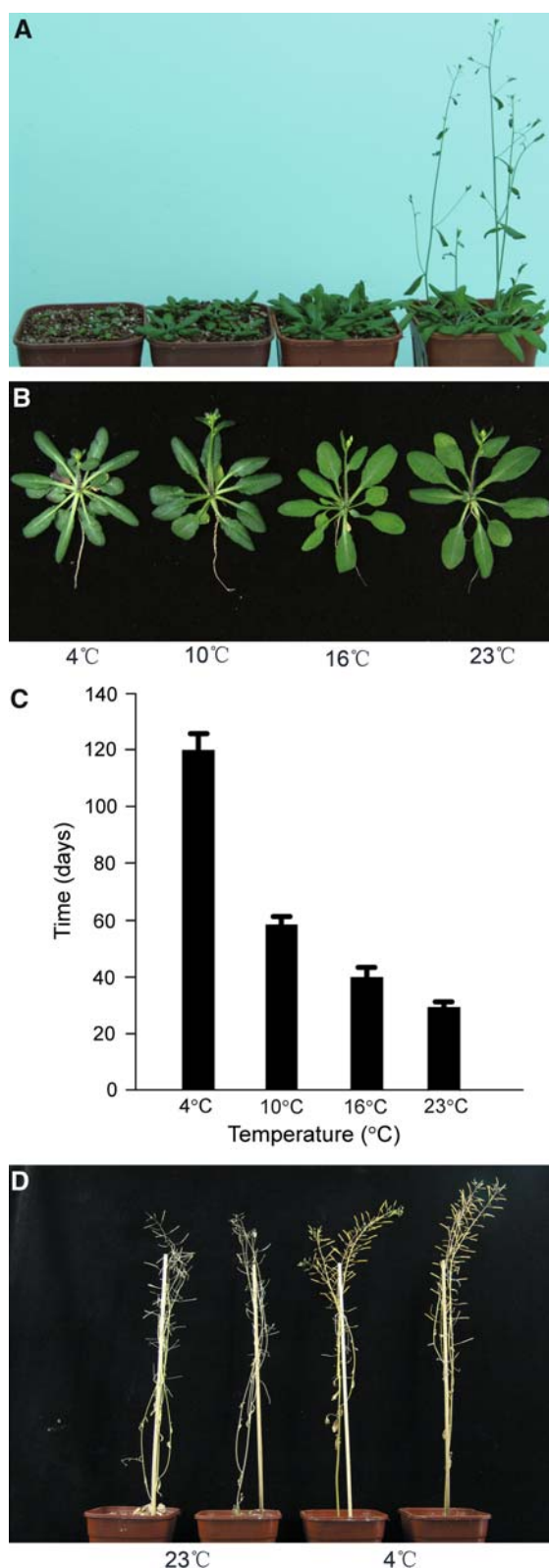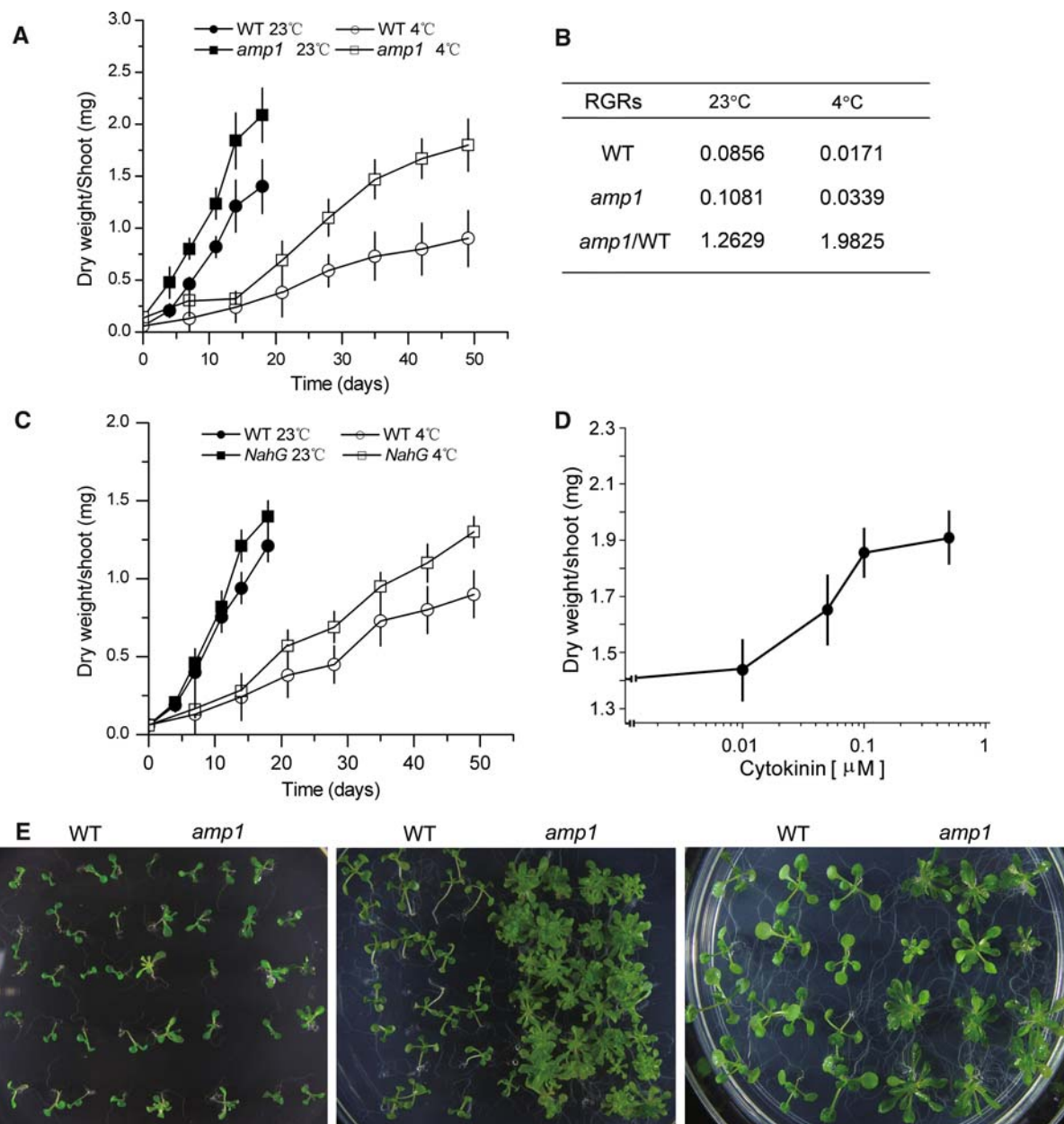
Fig. 2 Temperature effects on WT and *amp1* growth. **a** Mean shoot-plant biomass of WT and *amp1* plants during incubation at 4°C or during the same period at 23°C. **b** RGRs were estimated as the regression line gradient of in over periods of growth (*W* against *t*). **c** Mean shoot-plant biomass of WT and *NahG* plants during incubation at 4°C or during the same period at 23°C. **d** Mean average shoot-plants biomass under cytokinin treatment after 3 months at 4°C. **e** The phenotypic differences of wild-type and *amp1* plants at 23°C for 1 week (left) or for 3 weeks at 23°C (right) and after 1 month under 4°C treatment (middle)

whether elevated cytokinin levels might play a role in mediating the enhanced growth of *amp1* at 4°C, we treated wild-type seedlings with exogenous zeatin and tested their growth in the cold. Seedlings were grown at 23°C for 1 week and then were transferred to the 4°C chamber under zeatin treatment (0.01, 0.05, 0.1 and 0.5 μM) for 3 months, whereupon shoot dry weight was measured. Exogenous zeatin could enhance growth in the cold as effected as *amp1* (Fig. 2d). These results are consistent with the idea
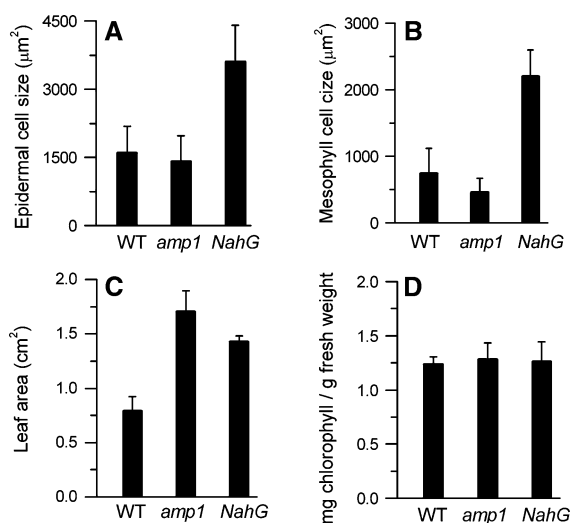
Fig. 3 Leaf growth of WT, *amp1* and *NahG* plants at 4°C. **a**, **b** Cross-sectional areas of epidermal cells or mesophyll cells from plants kept 50 days at 4°C. **c** Mean areas of fully expanded rosette leaves from plants kept 50 days at 4°C. **d** The chlorophyll content of WT, *amp1* and *NahG* plants kept 50 days under 4°C treatment

that cytokinin promotes growth under low temperature conditions.

Effect of salicylic acid levels on growth at low temperature

Previously, enhanced growth at low temperature was reported for a transgenic plant, transformed with a bacterial salicylic acid hydroxylase gene, *NahG*, and that contains reduced amounts of salicylic acid. Shoot growth in this line was reported to be indistinguishable from the wild-type at 23°C but significantly faster at 5°C and accompanied by larger cells in rosette leaves (Scott et al. 2004). Under our experiment condition, the growth of *NahG* plants was also better than that of wild-type plants at 4°C, being associated with larger cells (Figs. 2c, 3). These results suggest that decreasing salicylic acid levels enhance the ability of cells to expand in the cold.

Analysis of the expression level of cyclin D and *CBF* genes

Under low temperature treatment, plants induce a set of genes, presumably to acclimate to the cold. The pathway for cold-responsive gene expression has been most clearly elucidated in Arabidopsis
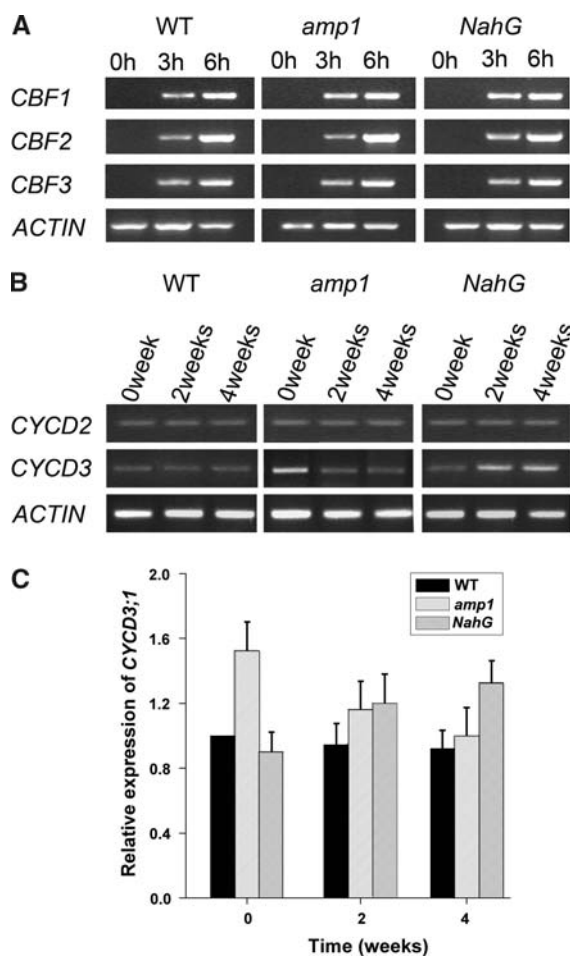
Fig. 4 Expression pattern of *CBFs* and *CYCD* genes in WT, *amp1* and *NahG* plants in response to cold stress. **a** Expression pattern of *CBF*1, *CBF*2 and *CBF*3. WT, *amp1* and *NahG* plants were grown at 23°C for 7 days and then cold treated at 4°C for 0, 3 and 6 h. **b** Expression pattern of *CYCD2;1* and *CYCD3;1*. WT, *amp1* and *NahG* plants were grown at 23°C for 7 days and then cold treated at 4°C for 0, 2 and 4 weeks. The *ACTIN* gene was used as a loading control. Five independent experiments were carried out and trends were similar. **c** Relative expression of *CYCD3;1* in (**b**)

(Thomashow 2001; Cook et al. 2004). A group of master transcription factors have been identified, termed CBFs, that between them appear to be responsible for activating most of the known, downstream, cold-responsive genes (Jaglo-Ottosen et al. 1998). Strikingly, the transcript levels of *CBF1, CBF2* and *CBF3* in wild-type, *amp1*, and *NahG* responded alike to low temperature (Fig. 4a), moreover, we found that expression levels of downstream, cold response genes (*RD29A, KIN1, KIN2, COR, RD*)

also had no difference (data not shown). Evidently, the improved performance of neither *amp1* nor *NahG* depends on altered *CBF* expression. As a further test of the role of *CBF* expression in plant growth at low temperature, we exposed a line that over-expresses *CBF1* to 4°C. Over 2 months of 4°C treatment, *CBF1* over-expressers grew more slowly and to a more limited extent than did the wild-type (data not shown). So, expression of *CBF1* is not promoting growth for the Arabidopsis plant's response to cold.

Under prolonged cold treatment, our results thus far suggest that the slow growth of the wild-type involves signaling from cytokinins decreasing or salicylic acid increasing, or both. It has been observed that cytokinin activated cell division in Arabidopsis through induction of a D-type cyclin, *CYCD3;1*, driving the G1/S phase transition (Riou-Khamlichi et al. 1999; Menges et al. 2006). Among the Arabidopsis *CYCD* genes, *CYCD2;1* and *CYCD3;1* are the best studied examples. We examined *CYCD2;1* and *CYCD3;1* expression at low temperature in wild-type, *amp1*, and *NahG* plants. For up to 4 weeks at 4°C, there was no difference between the genotypes in *CYCD2;1* transcript level (Fig. 4b). In contrast, *CYCD3;1* transcript level was higher in *amp1* than wild-type under control conditions (Fig. 4b, c). This suggests that the enhanced growth seen in *amp1* at 23°C may result from a faster cell cycle driven in part by the higher levels of this cyclin. However, during incubation at 4°C, *CYCD3;1* level in *amp1* decreased and became approximately equal to that of the wild-type. Thus promoted cell division, observed in *amp1* plants in the cold, did not correlate with the expression level of this gene in the cold. Factly, expression of *CYCD3;1* induced by cytokinin was detected obviously in seeding at two leaves stage (Riou-Khamlichi et al. 1999; Dewitte et al. 2003), and, it was possible that expression level of *CYCD3;1* was hard to detect in other stages, so expression level of the gene in *amp1* plants might be still higher than that in wild-type during long term cold treatment. Interestingly, *CYCD3;1* level increased with time in the cold in *NahG* plants though there was no difference at 23°C given their expansion-based promotion of growth at 4°C.

*NahG* plants display enhanced endoreduplication levels

The enhanced growth of *NahG* plants under low temperature treatment results from enhanced

expansion rather than from enhanced cell division (Scott et al. 2004). Endoreduplication is widespread in nature, and is thought to be driven by similar regulators as those that control the G1/S phase transition of the mitotic cell cycle. To determine whether the increased cell size of *NahG* under low temperature condition is associated with endoreduplication, we analyzed nuclear DNA content by flow cytometry. After 3.5 months at 4°C, wild-type leaves displayed a typical pattern, with C values (the haploid nuclear DNA content) ranging from 2 to 16C (Fig. 5a). This pattern was similar in *amp1* (Fig. 5b); however *NahG* plants had extensive endoreduplication (Fig. 5c, d). *NahG* plants had approximately one additional endocycle compared with wild-type plants, resulting in DNA values as high as 32C, and concomitantly the number of 2C cells was reduced. Given the well known correlation of cell size and ploidy levels (Melaragno et al. 1993), these results substantiate the pronounced cell expansion inferred for *NahG* transgenics following growth at low temperature.

Discussion

Low temperature is one of the most important environmental factors, which prolongs plant growth duration by lengthening the cell cycle. Regulation of the cell cycle duration seems to involve cytokinins as well as salicylic acid. We found that plant growth duration becomes longer as temperature decreases; but, even at 4°C, plants grew to maturity and ultimately attained the same size as plants growing under control conditions (23°C) (Fig. 1). These observations suggest that under our treatment, the growth of cells required a longer time to traverse the cell cycle but that the developmental program is not altered, and in this process, alteration of cytokinin and salicylic acid levels had important roles. In maize, seedlings given 4°C treatment during the night produced somewhat shorter leaves, accompanied by fewer cells, longer cell cycles but unchanged mature cell size (Rymen et al. 2007). These authors also identified some cell cycle regulators whose expression levels changed accordingly. We also found that the master, cold-responsive transcription factors (*CBF* genes) were induced by cold in both *amp1* and *NahG* plants with the same kinetics as in

Fig. 5 DNA ploidy level in WT, *amp1* and *NahG* plants. **a–c** Ploidy distribution of WT, *amp1* and *NahG* fully expanded leaves harvested 3.5 months at 4°C. **d** Quantification of the results shown in (**a**)
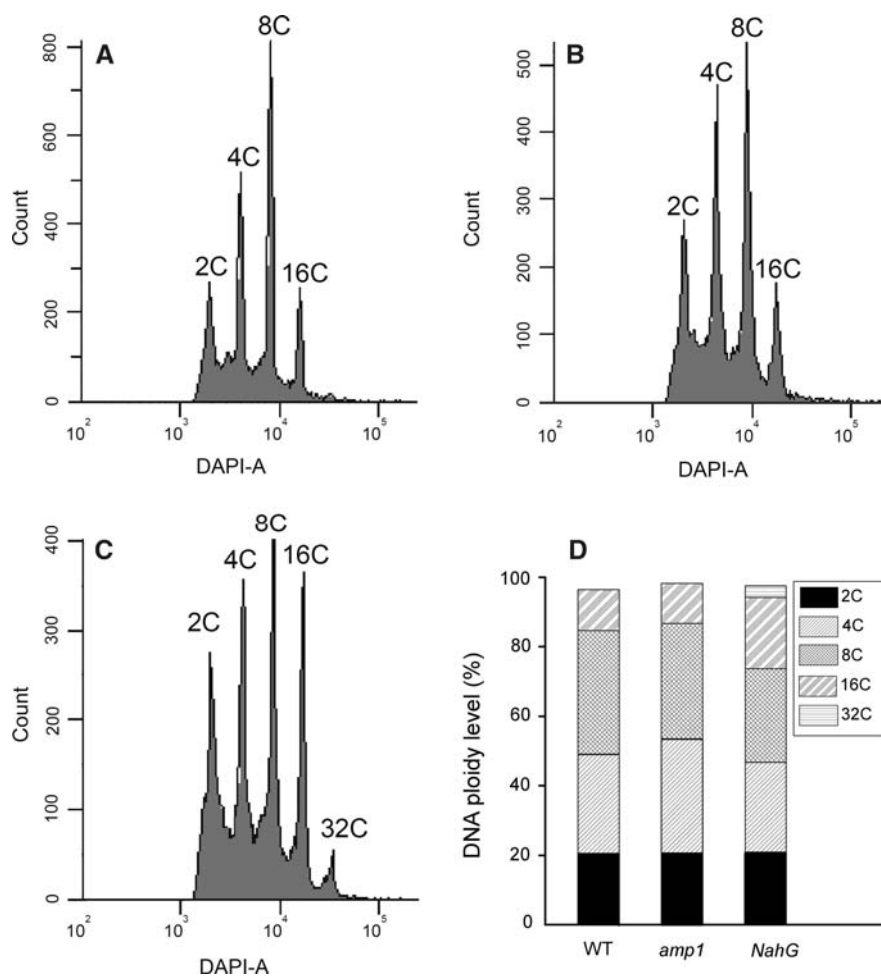
wild-type demonstrating that the improved growth of these lines at 4°C was not the result of higher or faster transcription of *CBF* genes and these mutant lines had capability to copy with low temperature.

Previously results have identified the G1/S phase transition as being an important checkpoint, limiting division at low temperature. In the *Allium cepa* root meristem, low temperature lengthened G1-phase and dropped the rate of progression across the G1/S transition by 25% (Giménez-Abián et al. 2004). Arrest in G1 phase, with the associated 2C amount of DNA, has been argued to bestow maximal protection on the genome in the presence of cold stress (Francis and Barlow 1988). Moreover, environmental stress including low temperature could affect checkpoints, delaying individual phase transition, which results in prolongation of cell cycle progression to provide time to repair damaged cellular components and to mount

anti-oxidant defense responses (Hartwell and Weinert 1989; Barnouin et al. 2002).

In Arabidopsis the D-type cyclin *CYCD3;1* promotes the G1/S phase transition (Menges et al. 2006), and is regulated by sucrose, hormones such as cytokinin (Riou-Khamlichi et al. 1999, 2000), auxin and gibberellin (Oakenfull et al. 2002) and, to a lesser extent, by brassinosteroid (Hu et al. 2000). Although previous studies have shown links between cytokinin, salicylic acid, and cell cycle in plant growth (Riou-Khamlichi et al. 1999; Vanacker et al. 2001), few data are available concerning the involvement of these compounds in the regulation of growth at low temperature. Here we analyzed the expression of two cyclin D genes and found that expression of *CYCD3;1* was correlated with the enhanced growth at 4°C, especially in *NahG* plants. *CYCD2;1* was constant under all conditions. *amp1* mutant had the high cytokinin level

caused by the increased size of the meristems (Helliwell et al. 2001), so *CYCD3;1* expression was still higher than that of wild-type at 4°C; and *CYCD3;1* expression increased in the *NahG* line at low temperature. Therefore, slightly higher *CYCD3;1* expression in *amp1* mutant at low temperature might allow to overcome the restrictions in G1/S transition, while elevated cytokinin content seemed to stimulate the G2/M transition, which resulted in higher cell division rate in this mutant in comparison with wild-type.

Endoreduplication is widespread in nature and its regulation plays an important role in plant growth and development. In plants, many organs comprise a mixture of cells of different ploidy levels, and this feature is prominent in hypocotyls and leaves (Melaragno et al. 1993; Gendreau et al. 1997). That plants can achieve an increased cell size through increasing their ploidy level by endoreduplication has been proposed to be part of a control mechanism that regulates cytoplasmic volume, adjusting it with respect to the DNA content of the nucleus (Kondorosi et al. 2000; Sugimoto-Shirasu and Roberts 2003). Phytohormones and temperature might control the exit from the cell cycle to endocycle during cell differentiation. Gibberellin has a global positive effect on endoreduplication and affected cell elongation in a concentration-dependent manner. Ethylene induces an extra round of endocycling acting on discrete ploidy levels. These observations suggest that endoreduplication initiates before elongation ends, and maintains growth potential for the subsequent elongation (Kieber et al. 1993; Gendreau et al. 1997, 1999). For *NahG* plants grown at low temperature, the enlarged cell size was associated with greater levels of endoreduplication, which correlated well with the stimulation of *CYCD3;1* expression. Endoreduplication is thought to be driven in part by some of the same regulators as control the G1/S phase transition of the mitotic cell cycle. In Arabidopsis, transcription level of *CYCD3;1* was associated with endoreduplication and cell size (Dewitte et al. 2003, 2007; Menges et al. 2006). In transformed plants over-expressing *E2Fb*, up-regulated expression of *CYCD3;1* was found with increased levels of endoreduplication (Sozzani et al. 2006). Moreover, low concentration salicylic acid also had affection on plant endoreduplication. When the salicylic acid pathway in *acd6-1-NahG* (accelerated cell death 6) plants were slightly reactivated with a low level of the *NahG*-insensitive salicylate agonist

BTH (benzo(1.2.3)thiadiazole-7-carbothioic acid), a few giant cells were found and the DNA content of the enlarged cells showed over 50% of nuclei with 16–64C or high, while wild-type showed a DNA content of 2–8C. At the same time, a partial block of salicylic acid signal transduction in *acd6-1npr1-1* (an *npr1-1* mutation partially blocks salicylic acid signaling) results in a similar activation of cell enlargement and endoreduplication. In contrast, high content level of salicylic acid could inhibit cell development (Vanacker et al. 2001). On the other hand, *npr1* mutant plants which had high level of salicylic aid grew worse than wild-type at low temperature (Scott et al. 2004). Here we postulated that low salicylic acid levels promoted to excessive cell enlargement by endoreduplication in the cold, and thus made large leaves.

In conclusion, long term exposure to low temperature prolonged cell cycle duration and resulted in a decreased plant growth. The alteration in cytokinin and salicylic acid levels may be parts of the pathway that regulates plant's response in this situation, which was not the result of higher or faster transcription of *CBF* genes. It is postulated that there is an increase in salicylic acid and decrease in cytokinin in plants at low temperature which may negatively regulate the plant growth via controlling the expression level of *CYCD3;1*.

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