NTNU – Trondheim
Norwegian University of
Science and Technology

Allyl isothiocyanate induces a shift in the cell cycle distribution of *Arabidopsis thaliana*

Signe Elisabeth Åsberg

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Supervisor: Atle M. Bones, IBI
Co-supervisor: Anders Øverby, IBI

Norwegian University of Science and Technology
Department of Biology

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Abstract

Isothiocyanates (ITCs) are degradation products of glucosinolates present in members of the Brassicaceae family acting e.g. as herbivore repellents and antimicrobial compounds. Recent results indicate that allyl ITC (AITC) has a role in defense responses such as glutathione depletion, ROS generation and stomatal closure. In this study we show that exposure to non-lethal concentrations of AITC causes a shift in the cell cycle distribution of *Arabidopsis thaliana* leading to accumulation of cells in S-phases and a reduced number of cells in C-phases. Furthermore, transcriptional analysis revealed an AITC-induced up-regulation of cyclin-dependent kinase A while several genes encoding mitotic proteins were down-regulated, suggesting an inhibition of mitotic processes. Interestingly, visualization of DNA synthesis indicated that exposure to AITC reduced the rate of DNA replication. Taken together, these results indicate that non-lethal concentrations of AITC induce cells of *A. thaliana* to enter the cell cycle and accumulate in S-phases, presumably as a part of a defensive response. Thus, this study indicates that AITC has several roles in plant defense and add evidence to the growing data supporting a multifunctional role of glucosinolates and their degradation products in plants.

Sammendrag

Isotiocyanater er degraderingsprodukter av glukosinolater som finnes i medlemmer av Korsblomstfamilien og er involvert i forvar mot herbivorer og pathogene mikroorganismer. Allyl ITC (AITC) har nylig blitt observert å ta del i andre forsvarsresponser slik som glutationdepresjon, generering av ROS og stenging av stomata. I denne studien viser vi at ikke-dødelige konsentrasjoner av AITC fører til et skift cellesyklusdstribusjonen til *Arabidopsis thaliana* som fører til en akkumulering av celler i S-faser og en reduksjon av antall celler i C-faser. Videre viser vi at AITC oppregulerer genuttrykket av cyclin-dependent kinase A og nedregulerer genuttrykket av flere gener for mitotiske proteiner, deriblant CDKB1;1. Dette indikerer en AITC-indusert inhibering av mitotiske prosesser. Visualisering av DNA syntese viste at AITC reduserte DNA replikasjon i cellene. Denne studien viser at ikke-dødelige konsentrasjoner av AITC induserer *A. thaliana* til å starte cellesyklusen og akkumulere i S-faser, antagelig som et ledd i en forsvarsrespons. I samsvar med andre nyere publikasjoner indikerer denne studien at AITC har flere roller i planteforsvar mot herbivorer og pathogene mikroorganismer.

Introduction

Glucosinolates are a group of secondary metabolites commonly found in members of the Brassicaceae family, including the cruciferous vegetables that have been part of the human diet for thousands of years (Halkier and Gershenzon, 2006). All known glucosinolate producing plants have at least one β-thioglucosidase often named myrosinase (Bones, 1996, Bones and Rossiter, 2006). Myrosinases hydrolyses glucosinolates into several potentially toxic compounds dependent on the reaction conditions and the presence of specifier proteins (Bones and Rossiter, 2006, Kissen et al., 2009a, Kissen et al., 2009b, Kissen and Bones, 2009, Kong et al., 2012, Halkier and Gershenzon, 2006, Wittstock and Burow, 2010). Glucosinolate degradation products are contributing to the distinct taste and flavor of cruciferous vegetables such as broccoli, mustard and wasabi and they constitute a potent defense system against herbivores and pathogens (Wittstock and Burow, 2010). To prevent constitutive production and potential damage to the plant cells, myrosinase is stored separately from its substrates in specialized cells called myrosin cells (Bones, 1985, Bones, 1996, Kissen et al., 2009b, Halkier and Gershenzon, 2006, Wittstock and Burow, 2010). The hydrolysis products are produced upon attack by herbivores or pathogens when damage to the plant tissue and distruption of the cells causes myrosinase to come into contact with glucosinolates (Bones, 1996, Bones and Rossiter, 2006, Kissen et al., 2009b, Halkier and Gershenzon, 2006, Wittstock and Burow, 2010).

The small sulfur-containing isothiocyanates (ITCs) are among the biodegradation products of glucosinolates. Due to their anticancer and chemopreventive properties, the ITCs have been the target of substantial research efforts over the last years (Cheung and Kong, 2009, Navarro et al., 2011). Little is known however of how ITCs affect plant cells or whether they have other physiological functions than as herbivore repellents or antimicrobials. Interestingly, several ITCs have been found to inhibit the growth of various plants species, including *A. thaliana* (Hara et al., 2010). Growth inhibition might partly be accounted for by ITC induced disruption of the microtubule network, as shown in a recent study by Øverby et al. to be inducible in *A. thaliana* by subjection to vapor phase of allyl ITC (AITC) (Øverby Anders, unpublished). Furthermore, AITC was found to induce stomatal closure in *A. thaliana* through a ROS dependent process (Islam et al., 2009, Khokon et al., 2011). Reduction of stomatal aperture is a much used defense strategy that prevents pathogen entry and

water loss upon herbivore attack, possibly suggesting a function for AITC in this commonly employed defense pathway (Khokon et al., 2011). Further support for a role of AITC in ROS mediated processes comes from recent results showing rapid depletion of intracellular gluthathione (GSH) and activation of gluthathione S-transferase genes in *A. thaliana* after AITC exposure (Øverby Anders, unpublished).

Although numerous studies have shown ITCs to interfere with the cell cycle progression of cancer cells, no studies have to our knowledge targeted the effect of ITCs on the plant cell cycle. Despite research efforts over the last decades, our understanding of the progression and regulation of the plant cell cycle remains limited (Francis, 2011). The plant cell cycle machinery differs in certain aspects from that of other eukaryotes, yet the main drives of the cell cycle in plants, yeast and mammals are the same: the highly conserved cyclin dependent kinases (CDKs) (Stals and Inze, 2001, Inze and De Veylder, 2006). CDKs are activated by complex formation with cyclins, the levels of which fluctuates throughout the cell cycle by regulated transcription and proteolysis (Inze and De Veylder, 2006, Boudolf et al., 2006). CDKA promotes the G1/S transition by binding CycD and subsequently phosphorylate the retinoblastoma related (RBR) protein. This activates the RBR/E2F/DP pathway, leading to DNA replication and progression into the G2 phase of the cell cycle (Inze and De Veylder, 2006). Cyclin-dependent kinase inhibitors (CKIs) have been suggested as the main negative regulators of the G2/M transition in plants (Boudolf et al., 2006, Francis, 2011). *A. thaliana* encodes seven ICK/KRPs that can bind CDKs and cyclins and the G2/M transition is likely to be driven by CDKB induced release of CDKA from ICK2/KRP2 (Inze and De Veylder, 2006, Boudolf et al., 2006, Verkest et al., 2005). It is however important to note that the possibility of CDKB directly driving the cell through G2/M has yet to be ruled out (Francis, 2007). Completion of the G2 intermediate phase is followed by mitosis and cytokinesis in which the chromosomes are separated and the cell divides.

Upon completion of cytokinesis, plant cells might progress to another round of DNA replication and cell division or enter the endocycle, an alternative cell cycle characterized by DNA replication without subsequent cell division (Inze and De Veylder, 2006). Endoreduplication is common to many plant species and is associated with the onset of differentiation and cell expansion (Inze and De Veylder, 2006,

Lammens et al., 2008). It has been suggested that inhibition of mitosis is sufficient for the cell to switch to the endocycle and correspondingly inhibitors of CDKs or cyclins such as the APC/C, WEE1, SIAMESE and ICKs/KRPs have been found to promote endocycle onset (Boudolf et al., 2004, Lammens et al., 2008, Inze and De Veylder, 2006). Interestingly, a recent study by Bao et al. linked cell cycle arrest to the onset of defense responses and expression of defense related genes (Bao et al., 2013).

In the present study, we investigate the effect of non-lethal, growth inhibiting concentrations of vapor phase of AITC on the cell cycle of *A. thaliana.* We show that AITC induces a distinct cell cycle shift resulting in increased S-phase populations in *A. thaliana* seedlings. Furthermore, we show that AITC reduces the expression of mitotic genes, indicating a potential regulation of the plant cell cycle by a metabolite commonly regarded as a feeding deterrent.

Results

AITC inhibits growth of A. thaliana seedlings and causes disintegration microtubules

To study the effect of AITC on the cell cycle of *A. thaliana* we employed 6-7 days old seedlings, a time when proliferation is still occurring throughout the first two true leaves (Skirycz et al., 2011). As cells of *A. thaliana* can take up to 48 hours to progress through the cell cycle, seedlings were exposed to AITC for 36, 48 and 60 h, thus ensuring enough time for all cells to go through the cell cycle at least once (Beemster et al., 2002). Exposure of 7 days-old seedlings of wild-type *A. thaliana* to vapor-phase AITC of 0, 10, 20 and 30 mM concentrations for 36, 48 and 60 h, revealed a dose-dependent growth inhibition consistent with previous reports (Fig. 1) (Hara et al., 2010, Øverby Anders). To study the severity of AITC-induced growth inhibition, seedlings were allowed to recover in an AITC free atmosphere for up to 11 days (Figure 1C) and seedling size was measured once a day during the first 4-6 days of recovery (Fig. 1B). AITC treated seedlings remained smaller than control seedlings throughout the recovery period, with seedlings exposed to 20 or 30 mM AITC showing the most significant growth inhibition. Concentrations of 1 M AITC has been reported to cause partial bleaching while 3.4 M AITC caused a substantial loss of chlorophyll and loss of green color (Øverby Anders, unpublished). In the present study, seedlings that were subjected to vapor of 20 and 30 mM AITC for 36 h displayed only bleaching of cotyledons at day 2 after treatment (Fig. 1C). Interestingly, seedlings that had been subjected to the same concentrations for 48 and 60 h displayed bleaching of cotyledons at the time when AITC exposure was stopped, although other chlorophyll containing organs remained green (Fig. 1C).

The microtubule cytoskeleton is important for cell division and expansion, both of which are required for plant growth and development (Liu et al., 2011). Exposure to vapor of 0.5 M and higher AITC concentrations has previously been shown to disintegrate microtubules in *A. thaliana* (Øverby Anders, unpublished). We therefore hypothesized that disintegration of the microtubule network might contribute to the observed growth inhibition after exposure to vapor of millimolar concentrations of AITC. To that end, a *A. thaliana* 35S::TUA-GFP line which expresses GFP-tagged α-tubulin was subjected to the same AITC treatments as above. Confocal microscopy of pavements cells of the first two true leaves revealed that all AITC treatments caused disintegration of the microtubule network (Fig. 2A). The seedlings used in this study

were subjected to AITC when 6 or 7 day-old, at which point cell division was still ongoing in most parts of the leaf. However, cells located at the tip of the leaf ceased dividing and began expanding in seedlings older than 8 days (Skirycz et al., 2011). Confocal microscopy showed that cells of AITC treated leaves were on average smaller than cells in non-treated leaves, indicating that AITC also prevented expansion of the cells (Fig. 2B).

AITC-induced cell cycle shift increases S-phase populations

Several studies have shown ITCs to induce cell cycle arrest in cancer cells, which is often found upon treatment with microtubule inhibitors (Zhang et al., 2003, Navarro et al., 2011). We therefore hypothesized that AITC-induced disintegration of the microtubule network prevented the cells from undergoing mitosis, resulting in cell cycle arrest, reduced growth rate and smaller *A. thaliana* seedlings. The distribution of cells in the cell cycle can be analyzed with flow cytometry. However, due to the size of plant cells and the presence of a rigid cell wall, extracted nuclei are used instead of whole cells. This results in the loss of cells that have proceeded beyond prophase where the nuclear envelope breaks down, preventing the flow cytometry analysis from distinguishing the G2/M phase of the cell cycle. Furthermore, *A. thaliana* inhabits endoreduplication, a process of repeated rounds of DNA replication that is not followed by cell division, resulting in nuclei with several copies of DNA. Therefore, the G2/M population is replaced with a population of cells containing 4 copies of DNA (4C). This population contains cells that will proceed either to mitosis, remain at 4C or enter the endocycle. Mitotic arrest will increase the population of 4C cells if the cells are arrested prior to nuclear envelope breakdown. However, mitotic arrest after nuclear envelope breakdown will result in a reduction of the 4C population. To investigate the effect of AITC on the cell cycle distribution of *A. thaliana,* 6-7 day-old seedlings were subjected to vapor of 0, 20 and 30 mM AITC for 36 h and 0, 10, 20 and 30 mM AITC for 48 and 60 h, followed by extraction of nuclei and analysis by flow cytometry. The analysis revealed that *A. thaliana* cells ranges from 2C-32C, consistent with previous reports (Fig. 3A) (Galbraith, 2009, Galbraith et al., 1991). Interestingly, AITC induced a significant dose-dependent decrease in the 4C population resulting in a reduction from 21.5 % to 18.5 % after 36 h AITC treatment (20 and 30 mM AITC) and from 27.1 % in the control to 24.8 % (10 mM), 22.6 % (20 mM) and 17.3 % (30 mM) after 48 h of AITC treatment (Fig. 3B).

Similarly, a decrease from 26.5 % in the control to 25.5 % (10 mM), 23.9 % (20 mM) and 19.2 % (30 mM) was seen after 60 h of AITC treatment. Even though this suggests a mitotic cell cycle arrest after nuclear envelope breakdown, a similar decrease was found in all C-phase populations. Furthermore, a statistically significant dose-dependent increase of all S-phase populations was observed at all time points. The increase was most prominent in the S1 population which for the 36 h treatment increased from 11.35 % in control seedlings to 14.5 % and 13.7 % for 20 and 30 mM AITC, respectively. After 48 h of AITC treatment the S1 population increased from 11.3 % in the control to 11.7 % (10 mM), 13.9 % (20 mM) and 17.4 % (30 mM). Similarly the S1 population increased from 11 % in control seedlings to 11.3 % (10 mM), 14.2 % (20 mM) and 19.9 % (30 mM) after 60 h of AITC treatment. Taken together, these results suggest that AITC, in a dose-dependent manner, induces a cell cycle shift towards larger S-phase populations and smaller C-phase populations in *A. thaliana*. Despite being evident for all cell cycle phases, this trend was most prominent for the 2C, S1, 4C and S2 phases which includes approximately 70 % of the cells.

Analysis of cell cycle related genes indicate inhibition of mitosis but not DNA synthesis

As shown by flow cytometry, AITC induced a cell cycle shift of increased S-phase populations while decreasing C-phase populations. To understand the role of cell cycle regulators in this shift we performed a quantitative polymerase chain reaction (qPCR) analysis of transcripts from 16 genes encoding proteins involved in cell cycle regulation (Tab. 1). For this analysis 6-7 day-old *A. thaliana* seedlings were subjected to vapor of 20 and 30 mM AITC for 36, 48 and 60 h, which induced the most significant cell cycle shifts. CDKA is the only CDK found to be active in the G1 and S phases in plant cells and it promotes the G1/S transition by complex formation with CycD and subsequent activation of the RBR/E2F/DP pathway (Inze and De Veylder, 2006). Interestingly, CDKA was found to be up-regulated after exposure to vapor of 20 and 30 mM AITC for 36 and 48 h, but was down-regulated after 20 mM for 60 h (Tab. 2). This indicates an initial activation of CDKA expression by 20 and 30 mM AITC that might promote entry of the cells into S-phases. Expression of other S-phase related genes included in this study (CycD1;1, CycD3;1 and CycD4;1) were too low to be reliably detected by our method. CDKA functions in coordination with CDKBs

to promote the transition from G2 to mitosis, possibly by CDKB1;1 induced release of CDKA from ICK2/KRP2 inhibition (Inze and De Veylder, 2006, Boudolf et al., 2006, Verkest et al., 2005). Exposure to vapor of 20 mM AITC for 36 h and 30 mM AITC for 48 h resulted in down-regulation of CDKB1;1. Expression levels of CDKB1;2 did not change, however CDKB2;1 was down-regulated by both doses after 36 h and CDKB2;2 was up-regulated after 48 h. Interestingly, none of the mitotic inhibitors included in this study were up-regulated, including ICK2/KRP2 (Tab. 2). However, the mitotic cyclins CycA1;1 and CycA2;3 were down-regulated by 20 mM AITC for 60 h and 30 mM for 48 and 60 h. Furthermore, CycA2;3 was down-regulated by 20 mM AITC for 36 h. The mitotic protein KNOLLE was also down-regulated, although not statistically significant for all treatments (Tab. 2). Taken together, these results indicate that AITC induces expression of S-phase related genes while down-regulating mitotic genes in *A. thaliana*.

Analysis of DNA synthesis following AITC-exposure

Increased S-phase populations could be the result of more cells undergoing DNA replication or be caused by increased initiation followed by failed completion of DNA replication in already existing cells. To study whether cells of AITC treated seedlings were able to replicate DNA, we employed an EdU cell proliferation assay (Invitrogen) that allows visualization of newly synthesized DNA (Fig. 4). Seedlings were subjected to vapor of 0, 10, 20 and 30 mM AITC for 36 and 48 h and incubated with EdU for 1 h. Seedlings that had been exposed to AITC displayed no DNA synthesis, while newly synthesized DNA was visible in control leaves. However, after subjection to 10 and 20 mM AITC for 36 h followed by EdU staining for 4 h, newly synthesized DNA was found in both AITC treated and control leaves (Fig. 4). Non-EdU treated seedlings were used as negative control and showed no new synthesized DNA regardless of incubation time. This indicates an AITC-induced reduction of DNA synthesis rate. Interestingly, after 4 h of EdU treatment, fewer of the leaves that had been exposed to vapor of 20 mM AITC for 36 h displayed DNA synthesizing cells than 10 mM treated- and control leaves. Taken together, these results suggests that AITC reduces the rate of DNA synthesis in a dose-dependent manner, although the interpretation of the assay was complicated by variation in the amounts of newly synthesized DNA in leaves from the same plants.

Discussion

ITCs are known to function as herbivore and pathogen deterrents, however recent studies suggests that these small metabolites have additional roles in plant defense. In the present study, we report that non-lethal concentrations of AITC induced a shift in the cell cycle distribution of *A. thaliana* leading to increased S-phase and decreased C-phase populations of cells. In a study by Hara et al. (2010) it was shown that concentrations of 10 and 100 mM AITC caused phytotoxicity in *A. thaliana* (Hara et al., 2010). However, in the present study vapor of 10 mM AITC for 36, 48 and 60 h did not cause any visible bleaching while 20 and 30 mM AITC caused bleaching of the cotyledons but not of the rosette leaves. These discrepancies are likely the result of different methods of AITC application. AITC is volatile and upon herbivore attack cells beyond the immediate wounding site are likely exposed to various concentrations of vapor phase AITC. Our application system models this form of AITC exposure, although all above ground organs are exposed to similar concentrations. The level of ITC exposure that plant cells encounter in nature is currently not known, although concentrations up to 100 mM has been suggested (Halkier and Gershenzon, 2006, Koroleva et al., 2000). However, as this estimate is based on the amount of glucosinolates contained in specialized cells it is unlikely that plant cells would be exposed to 100 mM ITCs (Hara et al., 2010). The physiological relevant concentrations of ITCs are likely to vary as different plant-herbivore or plant-pathogen interaction produces distinct collections of defensive metabolites that are often targeted specifically to the attacking herbivore or pathogen (Dixon, 2001, Clay et al., 2009, Bednarek et al., 2009, Bednarek and Osbourn, 2009). Determining what constitutes physiological relevant concentrations is further complicated by the spatial distribution of secondary metabolites involved in defense. Cells located close to the site of damage are likely exposed to other metabolites and higher concentrations than cells further away (Kliebenstein et al., 2005). In this study we exposed seedlings to low AITC concentrations that were non-lethal and only caused mild bleaching.

Several studies have shown ITCs to cause cell cycle arrest of cancer cells, yet to our knowledge this has not previously been shown for plants. Øverby et al. (Unpublished) has recently shown that AITC disintegrates microtubules in *A. thaliana.* To our surprise, despite inducing microtubule disintegration, AITC-exposure led to an increase of all S-phase populations suggesting an induction of DNA replication.

Transcriptional analysis of genes encoding cell cycle regulators showed an up-regulation of CDKA by 20 and 30 mM AITC for 36 and 48 h. Although a down-regulation was found after 60 h, this indicates that an initial response to AITC caused cells to enter the cell cycle. Furthermore, CDKB1;1 was down-regulated after exposure to vapor of 20 mM AITC for 36 h and 30 mM for 48 and 60 h. Interestingly, genes encoding several mitotic proteins were down-regulated by AITC exposure, including the mitotic cyclins CycA1;1 and CycA2;3 and the mitotic protein KNOLLE, which regulates cell plate formation during mitosis. The mitotic inhibitor ICK2/KRP2 has been suggested as an important regulator of the G2/M transition in plants. In this model ICK2/KRP2 binds and inhibits CDKA, thereby preventing progression into mitosis until CDKB1;1 induces the release of CDKA (Inze and De Veylder, 2006, Boudolf et al., 2006, Verkest et al., 2005). Interestingly, none of the mitotic inhibitors included in this study were up-regulated by AITC. In mammalian cells however, ITCs have been shown to bind proteins by reaction with cysteine sulfhydryl groups and thereby regulate protein activity (Cheung and Kong, 2009, Zhang, 2012). Similar events are likely to occur in plants cells and further attempts to elucidate the effects of AITC on mitotic processes, should therefore target the level and activity of mitotic inhibitor proteins. The AITC-induced up-regulation of CDKA and down-regulation of CDKB1;1 observed in this study could however suggest an induction of DNA synthesis followed by inhibition of mitotic entry. Interestingly, the mitotic inhibitor Wee1 was down-regulation by AITC treatment. As a part of the DNA damage check-point, Wee1 arrests the cell cycle by inhibition of CDKA and CDKBs when activated by DNA damage or certain stress conditions (Boudolf et al., 2006, Francis, 2007). The down-regulation of Wee1 suggests that these responses are not involved in the observed AITC-induced cell cycle shift in *A. thaliana*. Interestingly, genes encoding several mitotic proteins were down-regulated after AITC exposure (Tab. 1). These results indicate that AITC inhibits mitotic processes, however, this does not explain why cells accumulated in S-phases. Increased populations of cells in S-phases might be due to AITC induced DNA replication, but also to inhibition of DNA synthesis without affecting the onset of DNA replication. Although up-regulation of CDKA indicates AITC-induced entry into S-phase and the onset of DNA replication, the EdU assay showed that AITC treated cells had reduced rate of DNA synthesis. Taken together, these results suggest that AITC exposure induces the cells to enter the cell cycle and start DNA replication. Simultaneously, the

rate of DNA synthesis is reduced, causing increased S-phase populations and delayed seedling growth. Cell cycle arrest has previously been coupled to defensive responses. In a study by Bao et al. it was shown that cell cycle arrest induced defense responses and expression of defensive genes (Bao et al., 2013). Furthermore, cell cycle arrest was found to increase resistance to pathogens, although the link between cell cycle arrest and defense responses is currently not understood (Bao et al., 2013). It has been suggested that the chromosomal rearrangements that takes place during DNA replication and mitosis could make certain defense genes more available to transcription, however, more research is needed to elucidate the role of cell cycle arrest in defense (Bao et al., 2013). Furthermore, it needs to be elucidated if changes in the cell cycle distribution induced by ITCs or other secondary metabolites indeed improves the defense activity of plants and whether these responses occur in nature.

In conclusion, the present study shows that non-lethal concentrations of AITC induce a cell cycle shift in *A. thaliana*, causing cells to accumulate in S-phases. Previous reports show that the glucosinolate-myrosinase system has a role in plant defense against herbivores and pathogens (Clay et al., 2009, Bednarek et al., 2009, Bednarek and Osbourn, 2009), but a multifunctional role for the glucosinolate-myrosinase system has also been suggested (Bones et al., 1991). Our results add evidence to the growing data supporting a multifunctional role of glucosinolates and their degradation products in plants.

Materials and methods

Plant growth and AITC treatment

A. thaliana ecotype Columbia-0 (Col-0) wild-type and 35S::TUA-GFP seeds were disinfected by a chlorine/ethanol-based procedure and sowed on 9 cm petri-dishes with Murashige-Skoog (MS) agar (2.15 g/l NaCl, 20 g/l sucrose and 6 g/l 1:1 Bacto-Phtyo agar). The seeds were vernalized in the dark for 4 days at 4°C and subsequently incubated at room temperature under a 16/8 h light/dark cycle for 6-7 days. For AITC treatment, a 9 cm petri-dish with 6-7 days old plants (lid removed) were placed in a 13 cm petri-dish containing a filter paper to which 200 µl AITC diluted in rape seed oil (Landlord) was added. Rape seed oil without AITC was used as control. The petri-dish was sealed and the plants allowed to grow under the above-mentioned conditions for 36, 48 or 60 h. For analysis of the growth inhibitory effects of AITC the plant-containing petri-dish were removed from the larger petri-dish and plants allowed to recover under the above-mentioned growth conditions for 4-5 days. The seedlings were photographed and the software ImageJ was used to estimate seedling size.

Confocal microscopy of microtubules

6-7 days old seedlings of *A. thaliana* Col-0 35S::TUA-GFP were subjected to vapor of 0, 10, 20 and 30 mM AITC for 36, 48 and 60 h. The first two true leaves were then analyzed by confocal microscopy (Leica TCS SP5).

Flow cytometry analysis

For flow cytometry analysis 6-7 days old seedlings of *A. thaliana* Col-0 wild-type were subjected to vapor of 0, 10, 20 and 30 mM AITC for 36, 48 and 60 h. Roots were removed by scalpel and nuclei extracted from 20-70 mg of plant tissue by manual chopping with a razor blade for 2 min on a pre-cooled rack containing ice-cold Galbraith buffer (45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM MOPS, pH7 and 1 % Triton X-100) using 1.5 ml buffer/100 mg plant tissue. The homogenate was filtered through a 30 µm nylon filter and subjected to RNase A (100 µg/ml) for 30 minutes on ice. The homogenate was stained in the dark with propidium iodide (50 µg/ml) for 30 minutes on ice. Samples were stored for up to 4 h and vortexed for 5 sec prior to flow cytometry analysis. Care was taken to analyze the samples within 10 min of vortexing. The nuclei were analyzed with a Beckman-Coulter flow cytometer (Becton Dickinson LSR).

qPCR

For analysis of the expression of genes encoding cell cycle regulators, 6-7 days old seedlings of *A. thaliana* Col-0 wild-type were subjected to vapor of 0, 20 and 30 mM AITC for 36, 48 and 60 h. For tissue harvesting, roots were removed and seedlings snap-frozen in liquid nitrogen. RNA was extracted from ground tissue using Spectrum Plant Total RNA Kit. RNase-Free DNase Set was used to prevent DNA contamination and RNA concentration was measured with NanoDrop 1000 (Thermo Scientific). QuantiTec Reverse Transcription Kit was used for cDNA synthesis and qPCR performed with SYBRgreen in a 96-well plate in Lightcycler 480 (Roche Applied Science) with the following program: preincubation step of 95°C for 5 min, followed by 45 amplification cycles (95°C, 10 sec; 55°C, 10 sec; 72°C, 10 sec) and a melting curve analysis to check primer specificity. The housekeeping genes clathrin and TIP41 were used for normalization. Lightcycler 480 Software (Roche) was used to calculate cycle threshold values and LinRegPCR and REST 2009 (QIAGEN) were used to calculate PCR efficiencies and relative expression values. Primer sequences are given in supplementary material.

EdU assay

For analysis of DNA synthesis, 6-7 day-old seedlings of *A. thaliana* Col-0 wild-type were subjected to vapor of 0, 10, 20 and 30 mM AITC for 36 and 48 h prior to EdU incorporation using the Click-iT® EdU Alexa Fluor® 488 Imaging Kit. Seedlings were placed in eppendorf tubes with 500 µl 10 µM EdU (Invitrogen) and placed under the above-mentioned growth conditions for 1 and 4 h. After EdU incubation the EdU solution was removed and seedlings washed twice with 1 ml PBS followed by fixation in 1 ml 3.7 % formaldehyde in PBS for 1 h. Subsequently the fixative solution was removed and seedlings were washed twice with 1 ml 3 % BSA in PBS followed by incubation in 1 ml 0.5 % Triton X-100 in PBS for 20 min to increase the permeabilization of the cells. The permeabilization solution was removed and seedlings were washed twice with 1 ml 3 % BSA in PBS before removal of the washing solution. The seedlings were then incubated for 30 min in the dark in 500 µl Click-iT reaction cocktail prepared according to the manufacturer's instructions. Subsequently the seedlings were washed once in 1 ml 3 % BSA in PBS and the first two true leaves observed under the fluorescence microscope (Nikon Eclipse E800).

Statistical analysis

The means of flow cytometry data for each cell cycle phase were compared using one-way analysis of variance (ANOVA) with the Students-Newmans-Keuls post-hoc test. Equality of variance was tested with Lavene's test. If the assumption of equal variance was not fulfilled Kruskal-Wallis test with the appropriate post-hoc tests was used. All statistical analyses were done using MiniTab version 15 (www.minitab.com) and MedCalc for Windows version 12.4 (MedCalc Software, Mariakerke, Belgium).

Figures

Figure 1

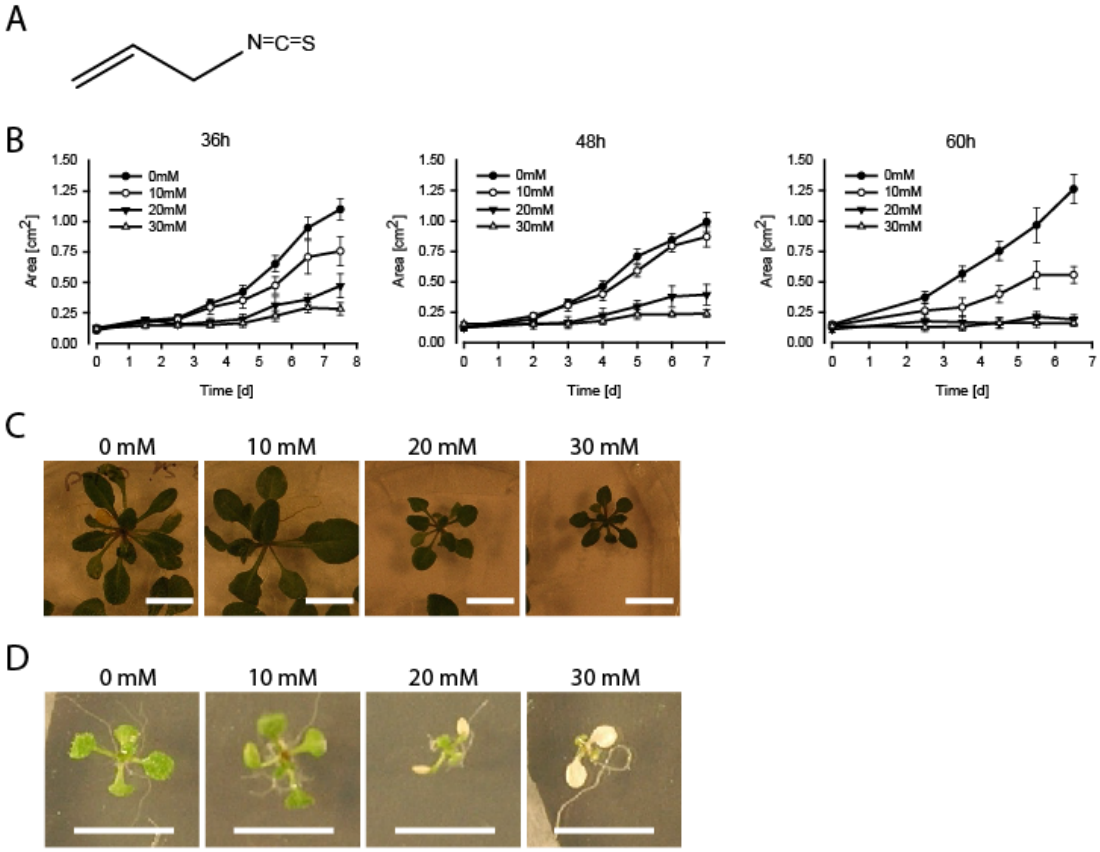

Figure 2

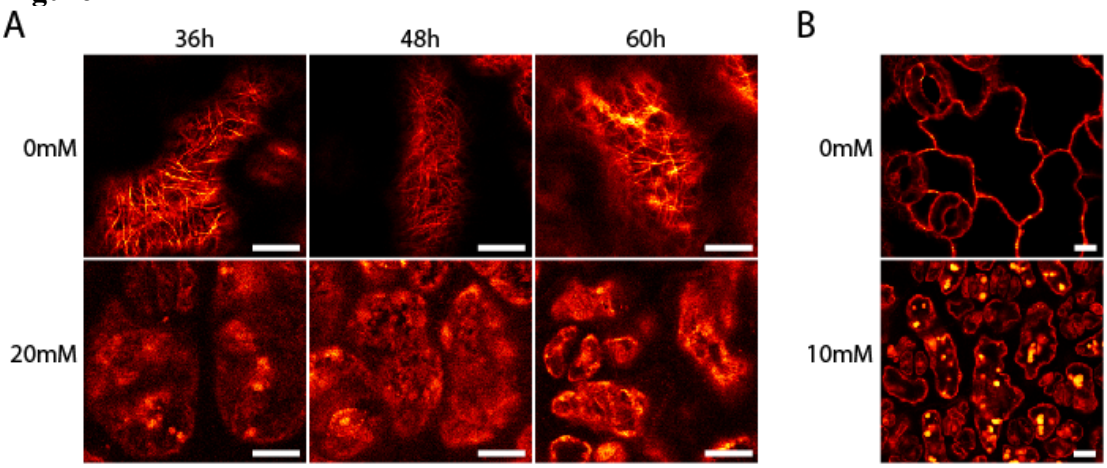

Figure 3

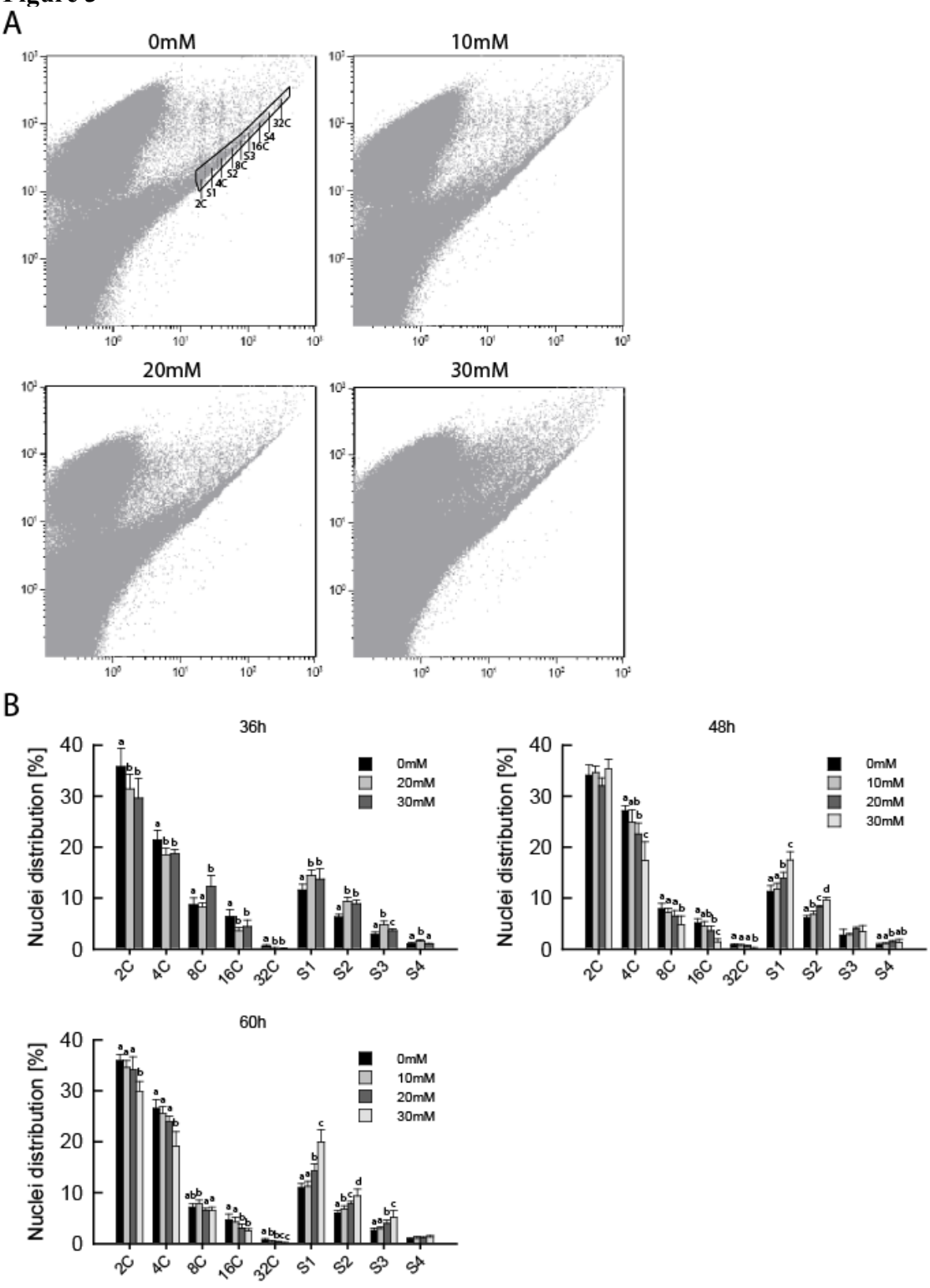
A
0mM
10mM
20mM
30mM
2C
S1
4C
S2
8C
S3
16C
S4
32C
B
36h
48h
60h
Nuclei distribution [%]
0mM
10mM
20mM
30mM
2C
4C
8C
16C
32C
S1
S2
S3
S4

Table 1

Gene	Accession number	Function
CDKA	AT3G48750	Regulates the G1/S and G2/M transitions
CDKB1;1	AT3G54180	Regulates the G2/M transition, ICK2/KRP2 inhibitor
CDKB1;2	AT2G38620	Regulates the G2/M transition
CDKB2;1	AT1G76540	Regulates the G2/M transition
CDKB2;2	AT1G20930	Regulates the G2/M transition
CycA1;1	AT1G44110	Active during G1/S and M, involved in DNA replication
CycA2;3	AT1G15570	Active during G1/S and M, negatively regulates endocycle
CycB1;1	AT4G37490	Mitotic cyclin regulating the G2/M transition
DEL1	AT3G48160	E2F transcription factors inhibits expression of CCS52A2 during mitosis
AtTCP15	AT1G69690	Transcription factor regulating expression of mitotic genes
KNOLLE	AT1G08560	Involved in cell plate formation
CCS52A2	AT4G11920	Subunit of APC/C, mitotic inhibitor
Wee1	AT1G02970	Mitotic inhibitor activated by DNA damage
ICK1/KRP1	AT2G23430	Inhibits mitosis by inactivating CDKs and cyclins
ICK2/KRP2	AT3G50630	Inhibits mitosis by inactivating CDKs and cyclins
SIAMESE	AT5G04470	Promotes entry into endocycle

Table 2

	20 mM						30 mM					
Gene	**36 h**		**48 h**		**60 h**		**36 h**		**48 h**		**60 h**	
CDKA	**4.3***	1.9 - 11.4	**2.0****	0.4 - 4.2	**0.3***	0.2 - 0.4	**5.5***	2.2 - 14.4	**3.7***	2.8 - 5.1	**0.5**	0.2 - 1.1
CDKB1;1	**0.2***	0.1 - 0.5	**0.6**	0.3 - 1	**0.7**	0.5 – 1	**0.8**	0.4 - 2.3	**0.1****	0.1 - 0.2	**0.2***	0.1 - 0.3
CDKB1;2	**1.4**	0.8 - 2.5	**0.8**	0.5 - 1.4	**0.5**	0.3 - 0.8	**1.7**	0.9 - 2.9	**1.8**	1.2 - 2.8	**0.7**	0.5 - 1
CDKB2;1	**0.1****	0.06 - 0.1	**2.4**	0.8 - 8.8	**3.4**	1 - 9.2	**0.1****	0.09 - 0.2	**1.0**	0.3 - 3.2	**1.5**	0.6 - 3.9
CDKB2;2	**0.7***	0.5 - 0.8	**1.7***	1.1 - 2.3	**1.0**	0.8 - 1.3	**0.8**	0.6 - 1.1	**1.8***	1.1 - 2.3	**1.0**	0.8 - 1.3
CycA1;1	**1.1**	0.7 - 1.8	**0.5**	0.2 - 1.1	**0.6***	0.5 - 0.8	**0.7**	0.4 - 1.2	**0.9***	0.8 - 1	**0.3***	0.2 - 0.7
CycA2;3	**0.6***	0.5 - 0.8	**0.8**	0.5 - 1.3	**0.6***	0.8 - 0.6	**0.5**	0.23 - 0.9	**0.3***	0.3 - 0.4	**0.3***	0.2 - 0.5
CycB1;1	**0.9**	0.7 - 1.1	**0.5**	0.2 - 0.8	**1.0**	0.8 - 1.2	**0.5**	0.3 - 0.8	**0.6**	0.3 - 1.2	**0.8**	0.3 - 1.4
DEL1	**1.2**	0.8 - 1.8	**0.7***	0.4 - 1.3	**0.5***	0.4 - 0.5	**0.1**	0.7 - 1.7	**1.1**	0.9 - 1.3	**0.5**	0.3 - 0.8
AtTCP15	**2.5**	1 - 5.2	**0.5**	0.2 - 0.8	**0.9**	0.6 - 1.1	**4.7**	3.8 - 6.3	**-**	-	**0.9**	0.3 - 4.4
KNOLLE	**0.6***	0.5 - 0.7	**0.7***	0.6 - 0.9	**0.8**	0.5 - 1.1	**0.6****	0.5 - 0.7	**0.7**	0.6 - 0.9	**0.7***	0.6 - 0.8
CCS52A2	**1.4**	1.3 - 1.7	**1.3**	1.2 - 1.4	**1.1**	1 - 1.1	**1.8**	1.6- 1.97	**1.7**	1.5 - 1.8	**1.3**	1.2 - 1.4
Wee1	**0.2***	0.1 - 0.5	**0.9**	0.7 - 1.4	**0.6**	0.4- 1	**0.7**	0.2 - 1.5	**0.3***	0.2 - 0.4	**0.2***	0.1 - 0.3
ICK1/KRP1	**0.7**	0.5 - 0.8	**1.9**	1 - 3.7	**1.2**	0.5 - 2.6	**1.0**	0.3 - 2.4	**1.5**	0.6 - 3.5	**0.9**	0.3 – 2.0
ICK2/KRP2	**2.2**	1.4 – 3	**0.7**	0.4 - 1.3	**1.0**	0.8 - 1.2	**1.8**	1.1 - 2.3	**1.4**	0.8 - 2.2	**0.8**	0.3 - 1.8
SIAMESE	**1.2***	1.1 - 1.2	**1.1***	1 - 1.2	**0.9**	0.9 - 1	**1.0**	0.9 - 1.1	**1.1**	1 - 1.2	**1.0**	0.9 - 1

Figure 4

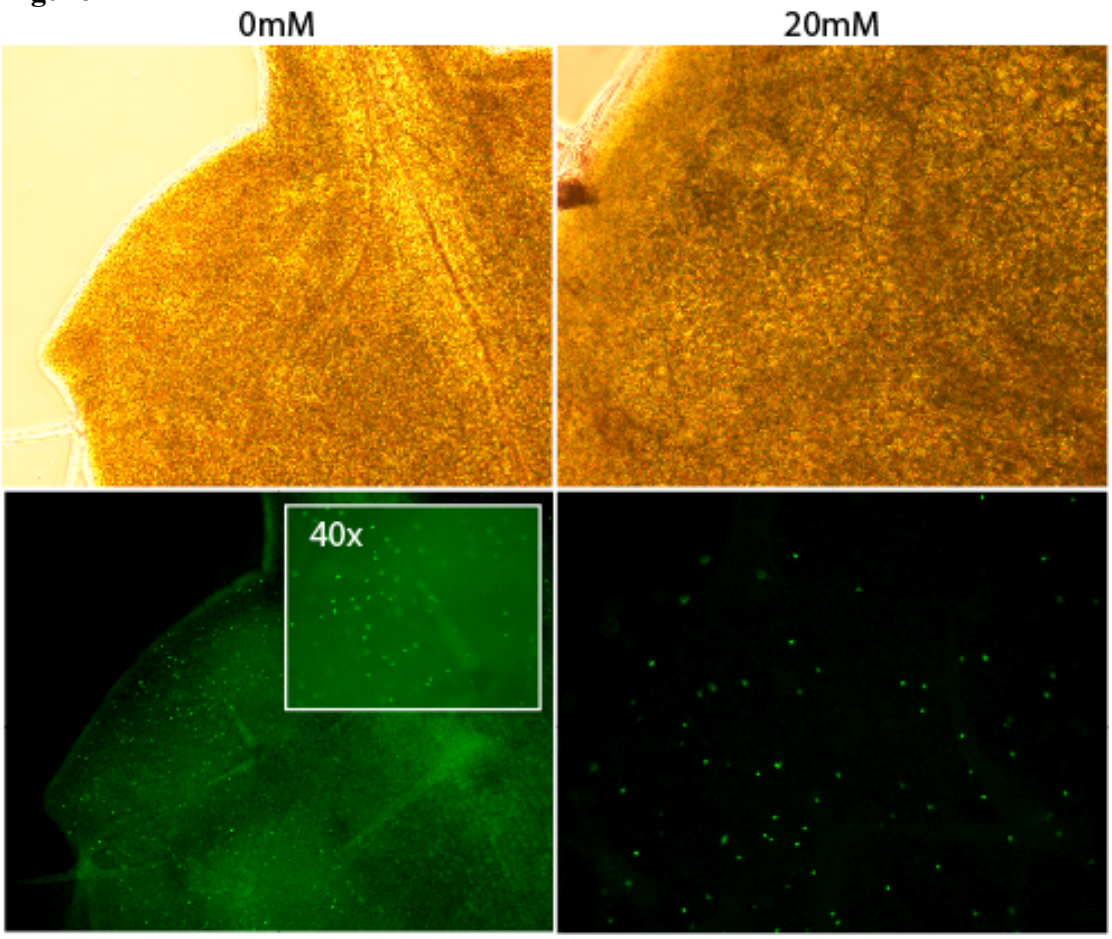

Figure legends

Figure 1 Growth inhibition and bleaching of *A. thaliana* seedlings subjected to AITC. A) Chemical structure of AITC. B-D) 7 day-old seedlings were subjected to vapor of 0, 10, 20 or 30 mM AITC for 36, 48 and 60 h and subsequently allowed to recover in an AITC free atmosphere for up to 11 days. B) Seedling size was measured once a day for 6-7 days. C) Dose-dependent growth-inhibition of seedlings subjected to 48 h of AITC treatment and 11 days of recovery. D) Bleaching of cotyledons after 48 h of AITC treatment. N=4-10 seedlings.

Figure 2 AITC disintegrates microtubules of *A. thaliana*. 6-7 day-old TUA-GFP seedlings were subjected to vapor of 0, 10, 20 or 30 mM AITC for 36, 48 and 60 h. The microtubule skeleton of pavement cells of the first two true leaves of two plants was analyzed by confocal microscopy for each treatment. A) Typical results of 20 mM AITC treatment are shown for all time points. B) Cells subjected to 10 mM AITC for 60 h are smaller than mock-treated cells. The bar is 10 μM.

Figure 3 AITC-induced cell cycle shift in *A. thaliana* causes increased S-phase populations and decreased C-phase populations. 6-7 day-old seedlings were subjected to vapor of 0, 10, 20 or 30 mM AITC for 36, 48 and 60 h and cell cycle distribution analyzed by flow cytometry. A) Flow cytometry plots obtained from seedlings subjected to AITC for 48 h. An example of gating is shown. B) Histograms obtained from flow cytometry plots show the percentage of nuclei in each cell cycle phase for all treatments. Different letters indicates statistically significant differences between treatments within the same cell cycle phase. The means of 3 experiments each with 2-3 replicates are shown.

Table 1 Cell cycle regulators selected for transcriptional analysis

Table 2 Transcriptional analysis of cell cycle related genes of *A. thaliana* subjected to AITC. Expression levels were determined by qPCR after subjection of *A. thaliana* seedlings to vapor of 0, 20 and 30 mM AITC of 36, 48 and 60 h. Data show point estimate and estimate calculated by REST of 3-4 replicates. Asterix indicates statistically significant difference from mock-treatment (*:P<0.05, **:P<0.07).

Figure 4 Newly synthesized DNA in cells of *A. thaliana* subjected to mock- or AITC-treatment. A) Newly synthesized DNA of a mock-treated seedling after 4 h of EdU staining. Insert is 40x from similar treatment showing newly synthesized DNA as bright spots. B) Newly synthesized DNA of a seedling subjected to 20 mM AITC for 36h and 4 h of EdU staining.

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Appendix

Table A1. Area of seedlings subjected to 0, 10, 20 and 30 mM of vapor phase AITC for 36, 48 and 60 h. Seedlings were photographed once a day and area measured by the software ImageJ. AITC treatment started at day 0 and stopped at day 1.

	36 h				48 h				60 h			
	10 mM	20 mM	30 mM	0 M	10 mM	20 mM	30 mM	0 M	10 mM	20 mM	30 mM	0 M
Day	Seedling area (cm)				Seedling area (cm)				Seedling area (cm)			
0	0.162	0.139	0.12	0.101	0.12	0.103	0.147	0.131	0.162	0.1	0.118	0.159
	0.111	0.12	0.075	0.098	0.06	0.122	0.194	0.097	0.163	0.091	0.114	0.127
	0.1	0.113	0.1	0.122	0.085	0.14	0.136	0.136	0.13	0.158	0.203	0.145
	0.039	0.129	0.164	0.098	0.159	0.11	0.167	0.108	0.062	0.096	0.127	0.121
	0.099	0.126	0.122	0.131	0.089	0.106	0.137	0.128	0.138	0.08	0.1	0.157
	0.116	0.2	0.102	0.136	0.14	0.103	0.169	0.149	0.154	0.117	0.131	0.08
	0.165	0.114	0.114	0.089	0.155	0.133	0.162	0.109	0.127	0.1	0.17	0.131
	0.106	0.12	0.097	0.08	0.124	0.089	0.172	0.145	0.105	0.125	0.137	0.148
	0.095	0.148	0.148	0.08	0.118	0.189	0.15	0.108	0.166	0.132	0.186	0.185
	0.078	0.116	0.117	0.081		0.121	0.208	0.111	0.093	0.204	0.147	0.175
1	0.202	0.299	0.154	0.175	0.216	0.134	0.143	0.189	0.348	0.265	0.156	0.425
	0.156	0.169	0.108	0.119	0.208	0.112	0.13	0.143	0.252	0.219	0.092	0.446
	0.038	0.144	0.166	0.183	0.119	0.203	0.161	0.197	0.248	0.131	0.176	0.328
	0.177	0.169	0.163	0.187	0.143	0.145	0.161	0.203	0.119	0.143	0.116	0.389
	0.177	0.195	0.128	0.184	0.218	0.105	0.155	0.209	0.334	0.102	0.105	0.429
	0.197		0.107	0.254	0.265	0.185	0.157	0.258	0.185	0.089		0.179
	0.233		0.114	0.178	0.199	0.146	0.135	0.212	0.129	0.221		0.334
	0.143		0.121	0.178	0.247	0.209	0.194	0.214	0.276	0.07		0.394
	0.126		0.142	0.156	0.271	0.097	0.134	0.269	0.141	0.146		0.469
	0.125		0.176	0.179	0.189	0.259	0.153	0.179	0.205	0.108		0.309
2	0.217	0.162	0.161	0.221	0.34	0.135	0.14	0.348	0.417	0.274	0.158	0.508
	0.212	0.125	0.135	0.162	0.128	0.088	0.165	0.332	0.497	0.214	0.084	0.459
	0.189	0.178	0.134	0.213	0.222	0.225	0.153	0.209	0.315	0.131	0.177	0.448
	0.066	0.148	0.179	0.171	0.317	0.144	0.146	0.326	0.338	0.17	0.134	0.546
	0.191	0.127	0.155	0.249	0.375	0.08	0.135	0.376	0.156	0.088	0.113	0.653
	0.205	0.368	0.131	0.303	0.364	0.199	0.16	0.466	0.22	0.115		0.272
	0.237	0.182	0.2	0.211	0.262	0.159	0.141	0.305	0.165	0.269		0.538
	0.152	0.16	0.143	0.202	0.335	0.206	0.231	0.329	0.316	0.094		0.578
	0.163	0.158	0.118	0.197	0.319	0.076	0.073	0.287	0.203	0.161		0.642
	0.144	0.231	0.169	0.243	0.189	0.278	0.132	0.275	0.234	0.123		0.496
3	0.322	0.202	0.17	0.329	0.363	0.16	0.168	0.476	0.702	0.429	0.204	0.802
	0.37	0.144	0.133	0.238	0.255	0.106	0.18	0.468	0.476	0.288	0.105	0.602
	0.268	0.192	0.221	0.345	0.291	0.263	0.26	0.274	0.499	0.164	0.199	0.647
	0.13	0.153	0.172	0.299	0.349	0.193	0.086	0.46	0.195	0.217	0.185	0.742
	0.362	0.135	0.146	0.365	0.416	0.09	0.171	0.599	0.685	0.129	0.126	0.871
	0.284	0.373	0.143	0.435	0.444	0.245	0.162	0.596	0.404	0.14		0.411
	0.368	0.223	0.111	0.335	0.32	0.162	0.169	0.517	0.317	0.326		0.698
	0.175	0.151	0.081	0.318	0.462	0.243	0.162	0.619	0.408	0.123		0.912
	0.226	0.191	0.15	0.322	0.438	0.087	0.178	0.382	0.328	0.214		0.807
	0.234	0.282	0.166	0.366	0.281	0.313	0.17	0.473	0.368	0.132		0.704

4	0.421	0.227	0.198	0.466	0.655	0.222	0.215	0.72	1.07	0.508	0.246	1.022
	0.317	0.156	0.16	0.323	0.337	0.306	0.229	0.356	0.962	0.396	0.132	0.799
	0.167	0.228	0.203	0.475	0.467	0.268	0.167	0.641	0.702	0.243	0.21	0.782
	0.416	0.154	0.18	0.447	0.563	0.118	0.23	0.711	0.736	0.234	0.169	0.937
	0.375	0.166	0.147	0.562	0.587	0.154	0.189	0.675	0.258	0.19	0.139	1.156
	0.434	0.407	0.144	0.603	0.617	0.316	0.209	0.823	0.509	0.192		0.663
	0.49	0.27	0.19	0.448	0.502	0.266	0.271	0.65	0.426	0.474		1.048
	0.244	0.175	0.102	0.38	0.632	0.337	0.315	0.778	0.508	0.161		1.304
	0.292	0.217	0.077	0.416	0.58	0.095	0.105	0.792	0.479	0.281		1.192
	0.287	0.305	0.199	0.505	0.414	0.375	0.199	0.543	0.539	0.195		0.982
5	0.604	0.279	0.218	0.672	0.974	0.258	0.21	0.88	1.085	0.581	0.256	1.224
	0.446	0.205	0.249	0.533	0.799	0.324	0.256	0.393	0.756	0.442	0.135	0.95
	0.261	0.308	0.317	0.628	0.392	0.292	0.167	0.809	0.658	0.224	0.199	0.961
	0.585	0.233	0.221	0.672	0.633	0.15	0.245	0.916	0.35	0.306	0.17	1.029
	0.501	0.246	0.212	0.777	0.785	0.165	0.208	0.82	0.878	0.196	0.142	1.421
	0.78	0.574	0.207	0.903	0.831	0.468	0.238	1.021	0.59	0.183		0.683
	0.683	0.287	0.14	0.514	0.704	0.41	0.235	1.031	0.48	0.148		1.199
	0.369	0.316	0.132	0.617	0.801	0.451	0.368	0.852	0.571	0.245		1.384
	0.435	0.432	0.262	0.741	0.85	0.115	0.148	0.751	0.485	0.156		1.236
	0.407	0.401	0.284	0.701	0.532	0.452	0.246	0.866	0.558			1.108
6	0.83	0.368	0.278	0.964	1.052	0.272	0.239	1.079				
	0.673	0.274	0.347	0.743	0.43	0.346	0.282	0.524				
	0.593	0.397	0.505	0.892	0.71	0.317	0.225	1.028				
	0.381	0.38	0.259	0.897	0.872	0.163	0.284	0.945				
	0.863	0.347	0.268	1.116	0.895	0.196	0.206	0.866				
	0.814	0.741	0.252	1.266	0.97	0.488	0.215	1.203				
	0.919	0.588	0.136	0.885	0.823	0.419	0.251	1.093				
	0.486	0.333	0.16	0.564	0.906	0.491	0.395	1.338				
	0.692	0.41	0.355	0.868	0.912	0.104	0.145	0.961				
	0.435	0.687	0.277	1.01	0.5	0.43	0.217	0.974				
7	0.892	0.428	0.257	1.088								
	0.628	0.283	0.218	0.91								
	0.428	0.398	0.336	1.029								
	0.848	0.444	0.493	0.958								
	0.78	0.397	0.255	1.232								
	1.015	0.88	0.251	1.564								
	1.022	0.668	0.293	0.779								
	0.591	0.43	0.141	1.042								
	0.799	0.539	0.171	1.188								
	0.514	0.87	0.366	1.023								

Table A2. Cell cycle distribution of *A. thaliana* cells after AITC-treatment as measured by flow cytometry. 6-7 day-old seedlings were subjected to vapor of 0, 10, 20 or 30 mM AITC for 36, 48 and 60 h and cell cycle distribution analyzed by flow cytometry. The percentage of nuclei in each cell cycle phase for all treatments are shown.

h	AITC (mM)	2C	S1	4C	S2	8C	S3	16 C	S4	32C
36	**0**	30.99	11.96	20.51	6.98	7.86	3.7	7.58	1.06	0.84
36	**0**	28.26	12.63	19.88	6.75	10.36	3.27	9.14	1.1	0.96
36	**0**	39.22	10.12	18.91	5.79	10.97	2.56	6.56	0.95	0.14
36	**0**	37.03	11.75	22.54	5.66	8.71	2.50	5.49	0.74	0.66
36	**0**	36.93	9.84	20.20	5.41	9.29	2.96	6.96	1.49	0.84
36	**0**	38.26	11.43	20.75	6.48	8.70	2.87	5.83	0.96	0.24
36	**0**	37.68	13.66	22.64	6.19	7.36	2.34	4.34	0.98	0.65
36	**0**	36.62	11.18	23.53	6.76	6.84	3.25	5.70	1.19	0.48
36	**0**	36.81	11.38	24.23	6.58	7.92	2.33	5.50	1.21	0.44
36	**20**	28.68	14.04	18.3	9.08	8.34	5.05	3.83	1.57	0.32
36	**20**	30.07	15.62	17.05	10.84	8.26	5.48	3.12	1.67	0.1
36	**20**	30.00	15.42	18.16	9.49	8.18	4.39	3.26	1.31	0.06
36	**20**	32.19	12.61	18.73	8.68	8.60	4.50	4.26	1.14	0.20
36	**20**	28.80	14.86	17.11	9.91	9.64	5.63	3.68	2.14	0.10
36	**20**	35.92	14.86	20.45	8.71	7.69	3.43	2.59	1.41	0.28
36	**20**	34.18	13.84	19.63	8.57	7.54	4.92	4.30	1.77	0.25
36	**30**	27.68	13.05	17.50	9.80	12.19	3.65	4.27	0.75	0.05
36	**30**	25.35	16.51	18.53	7.95	13.30	3.53	3.65	0.88	0.09
36	**30**	27.52	11.52	19.11	8.58	13.68	3.46	6.47	1.04	0.19
36	**30**	34.80	14.96	19.59	8.69	8.50	4.08	3.17	1.14	0.15
36	**30**	32.59	12.62	18.83	9.36	13.59	3.06	4.57	1.14	0.06
48	**0**	36.09	10.93	27.04	5.31	7.35	5.31	4.57	0.41	0.72
48	**0**	34.42	10.65	27.43	6.24	7.48	2.25	5.23	0.89	0.79
48	**0**	35.82	10.86	26.80	5.71	7.16	2.53	5.06	0.87	0.77
48	**0**	33.51	9.60	27.11	5.57	9.40	2.32	6.62	1.18	1.12
48	**0**	33.40	13.17	28.75	6.58	7.04	1.99	3.75	0.73	0.44
48	**0**	30.16	11.93	27.35	6.16	9.52	2.43	5.32	1.09	1.13
48	**0**	35.13	11.96	25.38	6.79	7.49	2.34	5.25	0.84	0.64
48	**10**	34.28	11.95	23.64	7.03	6.60	2.59	3.12	0.99	0.32
48	**10**	34.72	12.89	21.66	7.03	7.15	3.19	5.58	1.06	1.00
48	**10**	36.57	9.91	25.94	5.78	7.92	2.56	4.62	1.18	0.50
48	**10**	34.16	12.55	24.90	6.80	7.92	2.79	4.82	1.01	0.77
48	**10**	33.49	11.40	28.17	7.44	6.16	3.00	4.27	1.09	0.74
48	**20**	34.11	12.52	21.88	8.29	5.54	4.39	2.59	1.58	0.39
48	**20**	32.72	13.44	21.20	8.20	8.05	3.47	5.10	1.20	0.95
48	**20**	31.37	13.54	20.25	8.59	6.78	4.35	3.56	1.65	0.42
48	**20**	30.04	15.48	25.12	8.18	5.39	3.78	3.07	1.46	0.55
48	**20**	31.95	14.71	24.54	8.32	6.59	3.63	3.57	1.39	0.52
48	**30**	36.51	19.47	12.47	9.12	2.26	2.40	0.45	0.36	0.11
48	**30**	37.35	16.18	17.14	9.26	6.22	2.25	1.49	1.05	0.06
48	**30**	33.80	18.05	18.29	10.20	5.06	4.67	1.84	1.69	0.34
48	**30**	33.52	16.02	21.35	9.98	5.44	4.19	1.73	1.94	0.18

60	**0**	34.85	10.95	29.25	6.07	7.17	2.34	2.69	0.79	0.56
60	**0**	34.98	10.24	28.32	5.36	8.30	1.87	5.89	0.76	0.67
60	**0**	35.89	10.55	26.11	5.95	7.28	2.60	5.66	1.12	1.00
60	**0**	38.15	10.79	25.07	5.90	7.21	2.58	5.28	0.66	0.41
60	**0**	35.80	12.58	24.95	7.02	6.28	2.66	3.97	1.01	0.83
60	**0**	35.02	11.64	25.14	6.41	7.67	3.36	4.62	0.87	0.97
60	**0**	36.91	10.34	26.95	5.60	6.39	2.84	4.95	1.21	0.86
60	**10**	36.34	11.47	25.59	6.40	7.89	2.34	3.56	0.72	0.15
60	**10**	33.42	11.90	24.08	7.11	8.69	3.06	5.53	1.31	0.56
60	**10**	33.98	9.79	27.81	7.16	7.10	3.00	3.42	1.06	0.50
60	**10**	33.39	12.39	25.20	7.43	7.17	3.30	4.18	1.49	0.45
60	**10**	35.71	10.79	24.90	6.05	8.37	2.93	4.82	0.88	0.74
60	**20**	37.07	13.71	23.54	7.41	6.17	4.55	2.17	1.43	0.15
60	**20**	35.11	14.42	23.22	7.95	6.18	3.77	2.27	1.15	0.21
60	**20**	35.47	12.64	23.57	7.64	6.94	4.71	3.29	1.21	0.42
60	**20**	31.75	16.50	23.48	7.48	6.90	3.39	3.74	0.83	0.27
60	**20**	31.09	13.91	25.82	8.66	6.59	3.79	3.89	1.19	0.53
60	**30**	28.99	21.26	19.24	10.57	6.64	4.86	2.05	0.91	0.03
60	**30**	27.52	23.64	14.34	10.92	5.33	7.44	2.31	1.08	0.06
60	**30**	32.87	17.96	20.17	8.69	6.90	4.79	3.05	1.46	0.18
60	**30**	30.41	18.42	21.39	9.06	7.06	3.92	2.71	1.44	0.18
60	**30**	29.49	18.45	20.64	7.65	6.59	4.76	2.65	1.62	0.29

Table A3. Transcriptional analysis of cell cycle related genes of *A. thaliana* subjected to AITC. Expression levels were determined by qPCR after subjection of *A. thaliana* seedlings to vapor of 0, 20 and 30 mM AITC of 36, 48 and 60 h. Data show point estimate and estimate calculated by REST of 3-4 replicates.

	36 h					
	20 mM			30 mM		
Gene	**Expression**	**Std. Error**	**P(H1)**	**Expression**	**Std. Error**	**P(H1)**
CCS52A2	1.416	1.256 - 1.652	0.051	1.756	1.563 - 1.976	0
AtTCP15	2.525	0.970 - 5.019	0.263	4.65	3.842 - 6.316	0.058
CDKA	4.291	1.853 - 11.378	0	5.453	2.202 - 14.400	0.016
CDKB1;1	0.225	0.109 - 0.495	0	0.823	0.364 - 2.286	0.722
KNOLLE	0.582	0.455 - 0.680	0	0.56	0.463 - 0.719	0.07
CDKB1;2	1.418	0.765 - 2.495	0.361	1.687	0.921 - 2.861	0.228
CycA2;3	0.61	0.476 - 0.764	0	0.514	0.230 - 0.929	0.173
CDKB2;1	0.106	0.062 - 0.144	0.066	0.142	0.085 - 0.216	0.07
CycA1;1	1.107	0.670 - 1.843	0.75	0.69	0.401 - 1.180	0.37
Wee1	0.221	0.116 - 0.514	0	0.662	0.192 - 1.532	0.856
CycB1;1	0.9	0.738 - 1.106	0.538	0.48	0.254 - 0.750	0.051
DEL1	1.178	0.799 - 1.806	0.847	0.995	0.678 - 1.734	0.966
KRP1	0.645	0.531 - 0.833	0	1.019	0.337 - 2.369	0.905
CDKB2;2	0.681	0.533 - 0.826	0	0.792	0.573 - 1.107	0.335
SIAMESE	1.149	1.050 - 1.229	0.031	0.986	0.903 - 1.097	0.785
KRP2	2.231	1.401 - 3.012	0	1.749	1.078 - 2.322	0.01
	48 h					
	20 mM			**30 mM**		
	Expression	**Std. Error**	**P(H1)**	**Expression**	**Std. Error**	**P(H1)**
CCS52A2	1.314	1.196 - 1.412	0.018	1.659	1.498 - 1.807	0.032
AtTCP15	0.459	0.230 - 0.828	0.038			
CDKA	2.018	0.423 - 4.190	0.323	3.715	2.796 - 5.067	0.034
CDKB1;1	0.577	0.302 - 0.963	0.178	0.12	0.085 - 0.173	0.067
KNOLLE	0.736	0.625 - 0.861	0	0.728	0.605 - 0.894	0.14
CDKB1;2	0.82	0.450 - 1.432	0.63	1.783	1.205 - 2.747	0.17
CycA2;3	0.813	0.501 - 1.268	0.48	0.326	0.280 - 0.360	0
CDKB2;1	2.357	0.812 - 8.794	0.298	0.965	0.252 - 3.188	0.934
CycA1;1	0.488	0.187 - 1.054	0.112	0.854	0.748 - 0.960	0
Wee1	0.937	0.653 - 1.407	0.801	0.32	0.215 - 0.428	0.032
CycB1;1	0.491	0.241 - 0.755	0.029	0.559	0.274 - 1.160	0.3
DEL1	0.712	0.443 - 1.259	0.255	1.068	0.927 - 1.280	0.628
KRP1	1.869	0.959 - 3.703	0.214	1.481	0.598 - 3.453	0.519
CDKB2;2	1.687	1.122 - 2.326	0.051	1.757	1.122 - 2.280	0.034
SIAMESE	1.113	1.044 - 1.189	0.077	1.107	1.019 - 1.245	0.167
KRP2	0.702	0.443 - 1.278	0.429	1.443	0.782 - 2.242	0.398

	60 h					
	20 mM			30 mM		
	Expression	**Std. Error**	**P(H1)**	**Expression**	**Std. Error**	**P(H1)**
CCS52A2	1.053	1.000 - 1.115	0.24	1.287	1.185 - 1.397	0
AtTCP15	0.853	0.579 - 1.100	0.874	0.852	0.322 - 4.370	0.794
CDKA	0.297	0.214 - 0.417	0.034	0.474	0.185 - 1.112	0.288
CDKB1;1	0.733	0.494 - 1.019	0.232	0.209	0.136 - 0.336	0.008
KNOLLE	0.809	0.493 - 1.111	0.74	0.695	0.598 - 0.837	0.018
CDKB1;2	0.528	0.316 - 0.756	0.133	0.726	0.528 - 0.976	0.263
CycA2;3	0.546	0.457 - 0.615	0.023	0.307	0.202 - 0.490	0.034
CDKB2;1	3.43	1.015 - 9.210	0.2	1.48	0.647 - 3.933	0.64
CycA1;1	0.615	0.498 - 0.816	0.034	0.339	0.183 - 0.653	0.073
Wee1	0.623	0.378 - 1.022	0.27	0.203	0.129 - 0.347	0
CycB1;1	0.961	0.767 - 1.198	0.584	0.771	0.324 - 1.416	0.677
DEL1	0.454	0.350 - 0.531	0.034	0.518	0.318 - 0.794	0.12
KRP1	1.22	0.540 - 2.588	0.512	0.872	0.308 - 1.959	0.813
CDKB2;2	0.984	0.756 - 1.321	0.837	1.001	0.773 - 1.300	0.96
SIAMESE	0.931	0.863 - 1.009	0.255	0.956	0.880 - 1.039	0.412
KRP2	0.991	0.792 - 1.242	0.864	0.784	0.267 - 1.833	0.71