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CELL CYCLE MODULATION IN RESPONSE OF THE PRIMARY ROOT OF *ARABIDOPSIS* TO ABA

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Abstract

ABA plays a crucial role in plant growth and development in response to stress and it can inhibit root growth. Studies were carried out the importance of cell cycle regulation in mediating the primary root growth response of *Arabidopsis* to ABA. When seedlings were transferred to medium with ABA, the root growth rate and the *GUS* expression was progressively reduced with increasing concentrations of ABA. In addition, the expression levels of the *CDKA, CDKB1, CDKB2* genes roughly were constant, however, the *CYCB1* gene expression was down-regulated by ABA treatment. Our results indicated that the inhibition of primary root growth was mediated, at least partly, by an ABA-induced regulation of *CYCB1* expression at the G2/M checkpoint.

Introduction

Environmental stresses affect plant growth and development. Plants are sessile and unable to escape acute environmental stresses, they must develop sophisticated mechanisms to cope with and survive stress conditions. Abscisic acid (ABA) plays a crucial role in altering plant morphology in response to salt stress (Gurmani *et al*., 2007), heat stress (Liu *et al*., 2009), cold stress (Bano *et al*., 2009), oxidative stress (Xiong *et al*., 2006). Inhibition of primary root growth is a classic response mediated by ABA. Presence of ABA affects both cell extensibility and cell division during primary root growth (Finkelstein *et al*., 2002).

The positive and negative hormonal regulation of cell division has been reported. ABA reduced mitotic events in root meristem of *Arabidopsis* and sunflower (Robertson *et al*., 1990; Leung *et al*., 1994). Cytokinins caused accumulation of transcripts of cyclin A1 (Burssens *et al*., 2000a) and cyclin D3 (Riou-Khamlichi *et al*., 2000). JA interfered with G1/S and G2/M transition in synchronized BY-2 cells (Swiatek *et al*., 2002).

The cell cycle is normally described in terms of four distinctive phases: post- mitotic interphase (G1), DNA synthetic phase (S), post-synthetic interphase (G2) and mitosis (M-phase). Progression through the cell cycle is regulated at the G1/S and G2/M boundaries (Bryant & Francis, 1985) by protein kinases (Nurse, 1990) associated with regulatory proteins (cyclins) (Evans *et al*., 1983). In plants, two major classes of CDKs, known as A-type and B-type CDKs, have been studied to date. The A-type CDKs regulate both the G1-to-S and G2-to-M transitions, whereas the B-type CDKs seem to control the G2-to-M checkpoint only (Hemerly *et al*., 1995; Magyar *et al*., 1997; Porceddu *et al*., 2001).

Monomeric CDKs have no kinase activity and must associate with regulatory proteins called cyclins to be activated. Furthermore, the activity of the CDK/cyclin complexes is regulated by CDKs inhibitors (Dewitte & Murray, 2003). In addition, the phosphorylation state of various conserved residues of the CDK protein itself also determines its activity (Russo *et al*., 1996; De Veylder *et al*., 2003). A strong correlation between kinase activity and cell division activity in response to different environmental conditions has been reported in maize leaves (Granier *et al*., 2000), in wheat leaves under water stress (Schuppler *et al*., 1998), and in a comparative study of root growth of different Arabidopsis ecotypes (Beemster *et al*., 2002).

Multiple cyclins have been identified in *Arabidopsis* and they are classified into A-, B-, D-, and H-type cyclins, based mainly on sequence similarity (Vandepoele *et al*., 2002). B-type cyclins are subdivided into two subclasses, B1 and B2. In total, *Arabidopsis* contains nine B-type cyclins, of which four belong to the B1 class (CYCB1;1, CYB1;2, CYCB1;3 and CYCB1;4) and four belong to the B2 class (CYCB2;1, CYCB2;2, CYCB2;3 and CYCB2;4) (Vandepoele *et al*., 2002).

Nevertheless little is known about the mechanisms by which abiotic stress conditions affect the cell cycle regulation. B-type cyclins are good markers for cell proliferation as their expression patterns are specific to the G2/M phase of the cell cycle (Hemerly *et al*., 1992; Ferreira *et al*., 1994; Donnelly *et al*., 1999). Increasing experiments used *CYCB1* as a marker for cell proliferation. For example, *CYCB1* over-expression increases root elongation (Doerner *et al*., 1996). *CYCB1::GUS* expression was reduced in salt-stressed roots due to both decreased cell production and mature cell length (West *et al*., 2004), indicating severe disruption of mitotic activity.

Therefore we used a transgenic line (Designated A1) containing a *CYCB1::GUS* construct as a reporter line for mitotic activity to analyze the effect of ABA on cell cycle progression. The root growth rate and *GUS* gene expression was progressively reduced with increasing concentrations of ABA. In addition, the expression levels of the *CDKA, CDKB1, CDKB2* genes roughly were constant, however, the *CYCB1* gene expression was down-regulated by ABA treatment. These results indicate that the inhibition of primary root growth was mediated, at least partly, by an ABA-induced regulation of *CYCB1* expression at the G2/M checkpoint.

Materials and Methods

Plant Materials, growth conditions, ABA treatment and primary root length measurements: *Arabidopsis thaliana* seeds (ecotype Columbia) were surface-sterilised for 2 min in 75% ethanol, followed by 5 min in 1% NaClO solution and washed five times in sterile distilled water, plated on growth medium (MS medium, 1.5% sucrose, and 0.8% agar), vernalised at 4°C for 2 d in the dark and then exposed to white light. *Arabidopsis* plants were grown in a controlled growth room at 22 ± 2°C under long-day conditions (16 h light/8 h dark). For ABA application experiments, Seeds were sown on MS agar plates containing various concentrations of hormones and cultured vertically. The plates were scanned by an Epson perfection V200 Photo scanner and the primary root length of seedlings was measured by the tool Digimizer 3.2.1.0.

Gene expression analysis: The total RNA was extracted from 4, 6, 8-day-old seedlings grown on MS medium alone and MS medium supplemented with 0.4μ MABA with TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription (RT) was performed with an oligo (dT) primer. RNA (1μg) was heated at

70°C for 10 min and then immediately chilled on ice. RNA was then subjected to RT with reverse-transcriptase MMLV-RT SPCL (Invitrogen) at 42°C for 1 h according to the manufacturer's protocol. Synthesised cDNA was used as the PCR template. RT-PCR analyzed the transcript levels of marker genes (including *CDKA, CDKB1, CDKB2, CYCB1* and *CYCB2*).

Histochemical analysis and microscopy: For *GUS* staining, 8-day-old Seedlings grown on MS medium containing 0, 0.3, 0.4, 0.5 and 0.6 μ M ABA were stained for 2 hours at 37°C with *GUS*-staining solution (100 mM Na-phosphate buffer pH 7.0, 0.5 mM K4Fe (CN)6, 0.5 mM K3Fe(CN)6, 0.1% Triton X-100, 0.5 mg/ml XGluc), and subsequently destained in 95% ethanol. *CYCB1::GUS*-stained seedlings were mounted on slides with H_2O_2 for microscopic analyses. Photographs were taken with a light microscope (BX51, OLYMPUS, Tokyo). *CYCB1::GUS* expression of the 4, 6, 8-day-old seedlings grown on MS medium containing 0 or 0.4μ MABA were examined in the same manner.

Results

Inhibition of primary root growth is a classic response mediated by ABA. To test whether the effect was dosage-dependent, various concentrations (0, 0.3, 0.4, 0.5, and 0.6 μ M) of ABA were added to the medium. We determined the primary root growth by marking daily the position of the root tip on the plates. The ABA-sensitive response of *Arabidopsis thaliana* (Col-0) seedlings (*CYCB1::GUS* transgenic plant) occurred at concentrations as low as 0.3μ M ABA. When the ABA concentration was 0.6 μ M, the growth of A1 plants was arrested strongly (Fig. 1A and B). However, the molecular mechanism for ABA inhibition of plant growth is largely unknown. One of the possible mechanisms is exogenous application of ABA up-regulated ICK1(an homolog CDKs inhibitor p27Kip1) expression, which may lead to a block of G1/S transition (Wang *et al*., 1998).

As cell division and extension play an essential role in root growth and B-type cyclins are good markers for cell proliferation. So we used a transgenic reporter plant for cell proliferation expressing *CYCB1::GUS* (Colon-Carmona *et al*., 1999) to investigate the response of the primary root growth to ABA. In *Arabidopsis*, the *CYCB1* promoter is expressed upon entry into the G2-phase, and *GUS* staining patterns indicate regions containing cells engaged in active cell division (Colon-Carmona *et al*., 1999). *CYCB1::GUS* expression were gradually reduced with increasing concentrations of ABA (0, 0.3, 0.4, 0.5 and 0.6 μ M), particularly in 0.6 μ M ABA (Fig. 1 C). This finding suggests that, upon ABA treatment, the mitotic activity was disrupted to some degree. The kinematic analysis also explained the reduction of the *GUS* staining (Fig. 1D). The reduced *GUS* expression might be caused by a temporary or a long-standing inhibition of cell cycle. To investigate this possibility, we analyzed in more detail the *GUS* expression of A1 plants grown on MS containing 0.4μ M ABA at 4, 6 and 8 day. ABA did not apparently affect the density of *GUS* staining (Fig. 2A and B). However, staining zone in ABA treatment roots was smaller than that of the control roots, although the size of the zone of cells expressing the *GUS* gene was enlarged both in 0 and 0.4 μ MABA from fourth to eighth day. It is possible that ABA reduced cells engaged in cell division and the effect of inhibition is a long-standing.

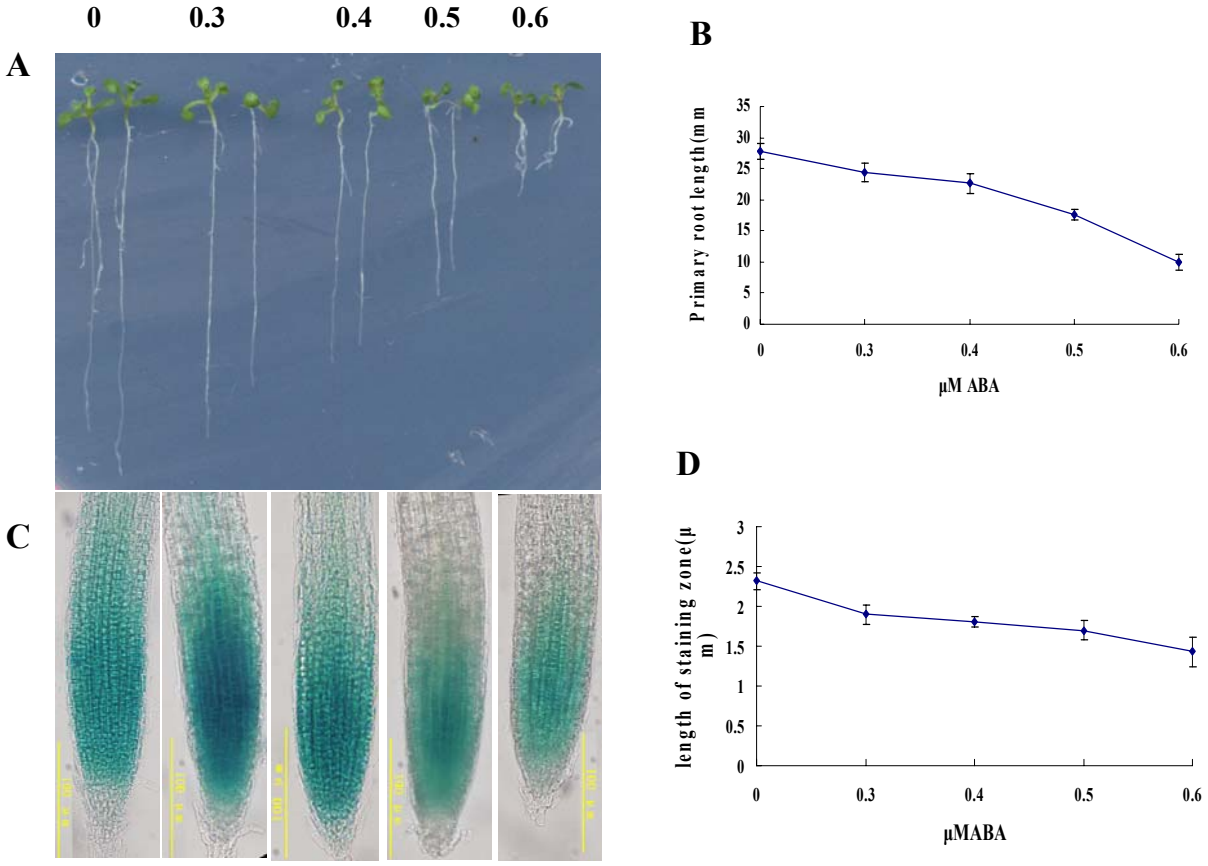

Fig . 1 ABA sensitivity of A1 plants.
A. Growth of A1 plants on MS medium containing a range of concentrations (0, 0.3, 0.4, 0.5 and 0.6 µ M) of ABA. Seeds were germinated for 8 d on MS medium with or without ABA, and representative plants were shown; B. ABA dose-response analysis of primary root length of A1 plants; C. The responding *GUS* staining of (A); D. Kinematic analyzed of the length of staining zone

We next sought to uncover the molecular events responsible for this change. The observation that the size of the region of stained cells had reduced in ABA treatment roots suggests the expression of cell cycle-regulated genes might be changed. In order to address this question, the expression levels of several cell cycle response genes were analysed by RT-PCR. 4, 6, 8 day-old seedlings grown on MS and MS containing 0.4µ MABA were chosen, RNA was prepared for RT-PCR. The transcript levels of the *CDKA, CDKB1, CDKB2* genes were roughly constant both in control and ABA treatment roots (Fig. 3), these results were consistent with fact that CDK activity is mainly regulated on a posttranslational level. Interestingly, it was found that the transcript of *CYCB1* was strongly up-regulated on sixth day and was lower with ABA treatment on fourth and sixth day. Surprisingly *CYCB1* nearly not expressed at eighth day without ABA treatment. Similarly expression of *CYCB2* was also up-regulated then recovered except which was not regulated by ABA (Fig. 3). These results support the view that CDK activity is mainly regulated on a posttranslational level, cyclin protein levels fluctuate in the cell cycle, cyclins could be a limiting factor for the mitotic activity of the CDK kinase (Ferreira *et al.*, 1994). Collectively, these results suggested that ABA might control primary root growth partly by regulating the expression of the *CYCB1*.

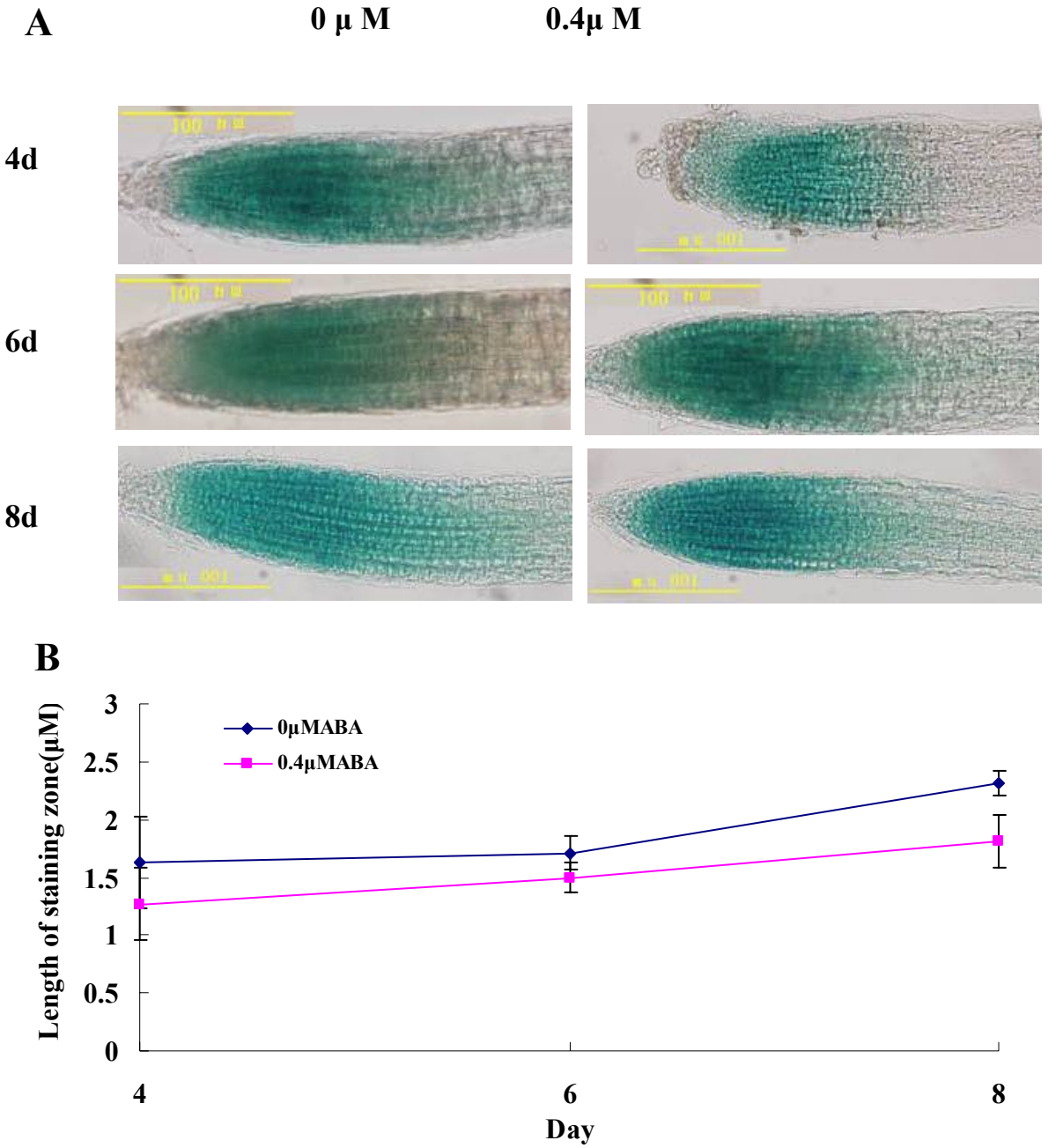

Fig. 2 CYCB1 promoter activity in the primary root tip of A1 plants
A. Seeds were germinated on MS medium containing 0 or 0.4μ MABA and histochemical *GUS*-staining were determined at different (4, 6, 8) days; B. Kinematic analyzed of the Length of staining zone

Discussion

Plant growth responses to environmental conditions have always intrigued physiologists. It is well known that adverse conditions inhibit root growth and that cell division and cell cycle regulation are involved in this response (Kurth *et al.*, 1986; Sacks *et al.*, 1997; Samarajeewa *et al.*, 1999; Burssens *et al.*, 2000b). The growth of the organ is the result of the production of new cells by cell division and their subsequent expansion (Bradford & Trewavas, 1994). The rate of cell production in a meristem is determined both by the number of dividing cells and the average time it takes to progress through the cell cycle.

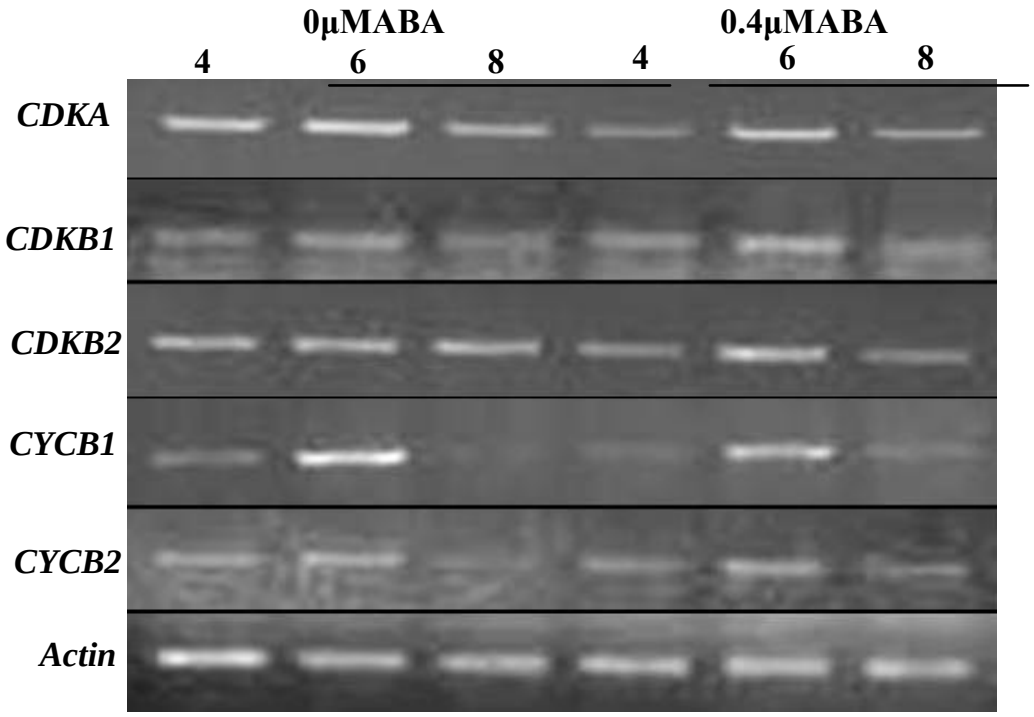

Fig . 3 Differential transcript levels of cell cycle regulatory genes in A1 plants.
4, 6, 8-d-old seedlings grown on MS medium containing 0 and 0.4μ MABA were chosen, Cell cycle response genes were analyzed.

Cell cycle regulators presumably control both cell cycle duration and the number of dividing cells. Presence of ABA affects both cell extensibility and cell division during primary root growth (Finkelstein *et al*., 2002). Our data showed that ABA inhibition of primary root growth was dosage-dependent. The effect was markedly when the ABA concentration was 0.6 μ M.

Cell division and extension play an essential role in root growth. It is possible that ABA control root growth through influence mitosis activity. In order to address this question, we analysed a transgenic line containing a *CYCB1::GUS* construct as a reporter of mitotic activity. It was found that ABA reduced *CYCB1::GUS* expression (Fig. 1C, D and Fig. 2). The CYCB1::GUS fusion protein is present only in cells in G2/M and is destroyed rapidly when cells passed through mitosis, so *GUS* staining patterns indicate regions containing cells engaged in active cell division. Thus these results indicate that cells engaged in mitosis were reduced.

To uncover the molecular events responsible for these changes, we also analysed the cell cycle primary-response genes *CDKA, CDKB1, CDKB2, CYCB1* and *CYCB2*. The transcript levels of the *CDKA, CDKB1, CDKB2* genes were constant but those of the *CYCB1, CYCB2* fluctuated in the cell cycle. The transcript of *CYCB1* and *CYCB2* genes were up-regulated on sixth day (Fig. 3). The difference was *CYCB1* gene was regulated by ABA but *CYCB2* was not. Our results suggest that ABA is involved in controlling G2/M progression during root meristem development.

Previous studies showed that ABA negatively influenced the cell cycle progression at the G1/S transition but not in the G2/M in tobacco BY-2 cells or during maize germination (Swiatek *et al*., 2002; Sanchez *et al*., 2005). Yin *et al*., (2009) reported that ABA did not apparently affect the expression of *CYCB1*. Our results showed that upon ABA treatment the transcript of *CYCB1* was also not obviously affected but it could be regulated by ABA (Fig. 3). It raised the possibility that ABA inhibition of primary root growth (Fig. 1A) was mediated, at least partly, by an ABA-induced regulation of *CYCB1* at the G2/M checkpoint. Previous studies have shown that ABA's general role in growth

inhibition maybe primarily linked to cyclin-dependent kinase inhibitors (CKIs) (Wang *et al*., 1997). However, it remains elusive how ABA regulates of cell division. Our results may help to provide new insights into the role of ABA in primary root growth.

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References

Bano, A., A. Rehman and M. Winiger. 2009. Altitudinal variation in the content of protein, proline, sugar and abscisic acid (ABA) in the alpine herbs from Hunza valley, Pakistan. *Pak. J. Bot*., 41(4): 1593-1602.

Beemster, G.T., K. De Vusser, E. De Tavernier, K. De Bock and D. Inze. 2002. Variation in growth rate between *Arabidopsis* ecotypes is correlated with cell division and A-type cyclin-dependent kinase activity. *Plant Physiol*., 129(2): 854-864.

Bradford, K.J. and A.J. Trewavas. 1994. Sensitivity thresholds and variable time scales in plant hormone action. *Plant Physiol*., 105(4):1029-1036.

Bryant, J.A. and D. Francis. 1985. *The Cell Division Cycle in Plants*. University Press, Cambridge.

Burssens, S., J.de Almeida Engler, T. Beeckman, C. Richard, O. Shaul, P. Ferreira, M. Van Montagu and D. Inze. 2000a. Developmental expression of the *Arabidopsis thaliana* CycA2; 1 gene. *Planta*, 211(5): 623-631.

Burssens, S., K. Himanen, B. van de Cotte, T. Beeckman, M. Van Montagu, D. Inze and N. Verbruggen. 2000b. Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in *Arabidopsis thaliana*. *Planta*, 211(5): 632-640.

Colon-Carmona, A., R. You, T. Haimovitch-Gal and P. Doerner. 1999. Technical advance: spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. *Plant J*., 20(4): 503-508.

De Veylder, L., J. Joubes and D. Inze. 2003. Plant cell cycle transitions. *Curr Opin Plant Biol*., 6(6): 536-543.

Dewitte, W. and J.A. Murray. 2003. The plant cell cycle. *Ann. Rev. Plant Biol*., 54: 235-264.

Doerner P., J.E. Jorgensen, R. You, J. Steppuhn and C.J. Lamb. 1996. Cyclin expression limits root growth and development. *Nature*, 380: 520-523.

Donnelly, P.M., D. Bonetta, H. Tsukaya, R.E. Dengler and N.G. Dengler. 1999. Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. *Dev. Biol*., 215(2): 407-419.

Evans, T., E.T. Rosenthal, J. Youngblom, D. Distel and T. Hunt. 1983. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*, 33(2): 389-396.

Ferreira, P.C., A.S. Hemerly, J.D. Engler, M. van Montagu, G. Engler and D. Inze. 1994. Developmental expression of the *Arabidopsis* cyclin gene cyc1At. *Plant Cell*, 6(12): 1763-1774.

Finkelstein, R.R., S.S. Gampala and C.D. Rock. 2002. Abscisic acid signaling in seeds and seedlings. *Plant Cell*, 14 Suppl: S15-45.

Granier, C., D. Inze and F. Tardieu. 2000: Spatial distribution of cell division rate can be deduced from that of p34(cdc2) kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. *Plant Physiol*., 124(3): 1393-1402.

Gurmani, A.R., A. Bano and M. Salim. 2007. Effect of abscisic acid and benzyladenine on growth and ion accumulation of wheat under salinity stress. *Pak. J. Bot*., 39(1): 141-149.

Hemerly, A., A. Engler Jde, C. Bergounioux, M. Van Montagu, G. Engler, D. Inze and P. Ferreira. 1995. Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. *EMBO J*., 14(16): 3925-3936.

Hemerly, A., C. Bergounioux, M. Van Montagu, D. Inze and P. Ferreira. 1992. Genes regulating the plant cell cycle: isolation of a mitotic-like cyclin from *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci*., 89(8): 3295-3299.

Kurth, E., G.R. Cramer, A. Lauchli and E. Epstein. 1986. Effects of NaCl and CaCl(2) on cell enlargement and cell production in cotton roots. *Plant Physiol*., 82(4): 1102-1106.

Leung, J., M. Bouvier-Durand, P.C. Morris, D. Guerrier, F. Chefdor and J.Giraudat. 1994. *Arabidopsis* ABA response gene ABI1: features of a calcium-modulated protein phosphatase. *Science*, 264(5164): 1448-1452.

Liu, T.X., Z.S. Zhang, J.B. Wang and R.Q. Li. 2009. Changes in abscisic acid immunolocalization in heat-stressed pepper seedlings. *Pak. J. Bot*., 41(3): 1173-1178.

Magyar, Z., T. Meszaros, P. Miskolczi, M. Deak, A. Feher, S. Brown, E. Kondorosi, A. Athanasiadis, S. Pongor, M. Bilgin, L. Bako, C. Koncz and D. Dudits. 1997. Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronized alfalfa cells. *Plant Cell*, 9(2): 223-235.

Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature*, 344(6266): 503-508.

Porceddu, A., H. Stals, J.P. Reichheld, G. Segers, L. De Veylder, R.P. Barroco, P. Casteels, M. Van Montagu, D. Inze, and V. Mironov. 2001. A plant-specific cyclin-dependent kinase is involved in the control of G2/M progression in plants. *J. Biol. Chem.,* 276(39): 36354-36360.

Riou-Khamlichi, C., M. Menges, J.M. Healy and J.A. Murray. 2000. Sugar control of the plant cell cycle: differential regulation of *Arabidopsis* D-type cyclin gene expression. *Mol. Cell Biol*., 20(13): 4513-4521.

Robertson J.M., K.T. Hubick, E.C.Yeung and D.M. Reid. 1990. Developmental responses to drought and abscisic acid in sunflower roots. *J. Exp. Bot*., 41: 339-350.

Russo, A.A., P.D. Jeffrey and N.P. Pavletich. 1996. Structural basis of cyclin-dependent kinase activation by phosphorylation. *Nat. Struct. Biol*., 3(8): 696-700.

Sacks, M.M., W.K. Silk and P. Burman. 1997. Effect of water stress on cortical cell division rates within the apical meristem of primary roots of maize. *Plant Physiol*., 114(2): 519-527.

Samarajeewa, P.K., R.A. Barrero, C. Umeda-Hara, M. Kawai and H. Uchimiya. 1999. Cortical cell death, cell proliferation, macromolecular movements and rTip1 expression patterns in roots of rice (*Oryza sativa* L.) under NaCl stress. *Planta*, 207: 354-361.

Sanchez, M.P., S.H. Gurusinghe, K.J. Bradford and J.M. Vazquez-Ramos. 2005. Differential response of PCNA and Cdk-A proteins and associated kinase activities to benzyladenine and abscisic acid during maize seed germination. *J. Exp. Bot*., 56(412): 515-523.

Schuppler, U., P.H. He, P.C. John and R. Munns. 1998. Effect of water stress on cell division and cell-division-cycle 2-like cell-cycle kinase activity in wheat leaves. *Plant Physiol*., 117(2): 667-678.

Swiatek, A., M. Lenjou, D. Van Bockstaele, D. Inze and H. Van Onckelen. 2002. Differential effect of jasmonic acid and abscisic acid on cell cycle progression in tobacco BY-2 cells. *Plant Physiol*., 128(1): 201-211.

Vandepoele, K., J. Raes, L. De Veylder, P. Rouze, S. Rombauts and D. Inze. 2002. Genome-wide analysis of core cell cycle genes in *Arabsidopsis*. *Plant Cell* , 14(4): 903-916.

Wang, H., L.C. Fowke. and W.L. Crosby. 1997. A plant cyclin-dependent kinase inhibitor gene. *Nature*, 386(6624): 451-452.

Wang, H., Q. Qi, P. Schorr, A.J. Cutler, W.L. Crosby and L.C. Fowke. 1998. ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant J*., 15(4): 501-510.

West, G., D. Inze and G.T. Beemster. 2004. Cell cycle modulation in the response of the primary root of Arabidopsis to salt stress. *Plant Physiol*., 135(2): 1050-1058.

Xiong, Y.C., G.M. Xing, C.M. Gong, F.M. Li, S.M. Wang, Z.X. Li and Y.F. Wang. 2006. Dual role of abscisic acid on antioxidative defense in grass pea seedling (*Lathyrus sativus* L.). *Pak. J. Bot*., 38(4): 999-1014.

Yin, H., X. Zhang, J. Liu, Y. Wang, J. He, T. Yang, X. Hong, Q. Yang and Z. Gong. 2009. Epigenetic regulation, somatic homologous recombination, and abscisic acid signaling are influenced by DNA polymerase epsilon mutation in Arabidopsis. *Plant Cell*, 21(2): 386-402.

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