

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/106044/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Cui, Weina, Wang, Hetong, Song, Jie, Cao, Xia, Rogers, Hilary, Francis, Dennis, Jia, Chunyun, Sun, Lizong, Hou, Meifang, Yang, Yuesuo, Tai, Peidong and Liu, Wan 2017. Cell cycle arrest mediated by Cd-induced DNA damage in Arabidopsis root tips. *Ecotoxicology and Environmental Safety* 145 , pp. 569-574. 10.1016/j.ecoenv.2017.07.074 file

Publishers page: <http://dx.doi.org/10.1016/j.ecoenv.2017.07.074>
<<http://dx.doi.org/10.1016/j.ecoenv.2017.07.074>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



1 Cell cycle arrest mediated by Cd-induced DNA damage in 2 Arabidopsis root tips

3 Weina Cui^{1,2}, Hetong Wang^{#,2,3}, Jie Song^{2,4}, Xia Cao^{2,5}, Hilary J Rogers⁶, Dennis Francis⁷, Chunyun Jia², Lizong
4 Sun², Meifang Hou¹, Yuesuo Yang⁷, Peidong Tai², Wan Liu^{2*}

5
6 ¹ Shanghai Institute of Technology, Shanghai 201418, PR China

7 ² Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese
8 Academy of Sciences, Shenyang 110016, P.R. China

9 ³ Department of Basic Medicine, He University, Shenyang 110163, P.R. China

10 ⁴ Environmental Science College, Liaoning University, Shenyang 110036, PR China

11 ⁵ Agricultural College, Shenyang Agricultural University, Shenyang 110161, P.R. China

12 ⁶ Cardiff University, School of Biosciences, Cardiff CF10 33TL, UK

13 ⁷ Key Laboratory of Eco-restoration, Shenyang University, Shenyang 11044, P.R. China

14 #Co-first author

15 * Corresponding author: Wan Liu; Tel: +86-24-83970367; Fax: +86-24-83970300; Email: liuwan63@hotmail.com

16

17 Abstract:

18 Accumulating evidence demonstrates that the aberrant expression of cell cycle
19 regulation and DNA repair genes can result in abnormal cell proliferation and genomic
20 instability in eukaryotic cells under different stresses. Herein, *Arabidopsis thaliana*
21 (*Arabidopsis*) seedlings were grown hydroponically on 0.5×MS media containing
22 cadmium (Cd) at 0–2.5 mg·L⁻¹ for 5 d of treatment. Real time quantitative reverse chain
23 reaction (qRT-PCR) analysis revealed that expression of DNA damage repair and cell
24 cycle regulation genes, including *BRCA1*, *MRE11*, *WEE1*, *CDKA;1* and *PCNA1*,
25 showed an inverted U-shaped dose-response. In contrast, notably reduced expression
26 was observed for G1-to-S transition-related genes, *Histone H4*, *E2Fa* and *PCNA2*;
27 DSB end processing, *GR1*; and DNA mismatch repair, *MSH2*, *MSH6* and *MLH1* genes
28 in root tips exposed to 0.125-2.5 mg L⁻¹ Cd for 5 d. Flow cytometry (FCM) analysis
29 revealed significant increases of cells with a ²C nuclear content and with a ⁴C and ⁸C
30 nuclear content under Cd stresses of 0.125 and 1-2.5 mg·L⁻¹, respectively. Our results
31 suggest that 0.125 mg·L⁻¹ Cd-induced DNA damage induced the marked G1/S arrest,

32 leading to accelerated growth in root tips, while 1.0-2.5 mg·L⁻¹ Cd-induced DNA
33 damage caused a notable G2/M arrest in root tips, leading to reduced growth in root
34 tips. This may be a protective mechanism that prevents cells with damaged DNA from
35 dividing under Cd stress.

36 **Key words:** Arabidopsis; Cd stress; DNA damage marker genes; cell cycle regulation
37 genes; Gene expression; Cell cycle arrest

38

39 **Abbreviations:**

40 CDKs Cyclin-dependent kinases
41 *CYCB1*;1 Cyclin B1;1
42 qRT-PCR Real time quantitative reverse chain reaction
43 *BRCA1* Breast cancer susceptibility1
44 *PCNA* Proliferation cell nuclear antigen
45 DSB double strand break
46 *GR1* Gamma response1
47 *MSH2* MutS homologue 2
48 *MLH1* MutL homologue 1
49 FCM Flow cytometry
50 ATM Ataxia-telangiectasia mutated
51 ATR Ataxia-telangiectasia and Rad3-related
52 ROS Reactive oxygen species

53

54 **Introduction**

55 Cadmium (Cd) is a highly persistent and accumulative heavy metal^s, and has been
56 listed as one among the top ten hazardous substances by the Agency for Toxic
57 Substances and Disease Registry (<http://www.atsdr.cdc.gov/cercla/07list.html>) and by
58 the National Toxicology Program (NTP 2004). Cd is ubiquitously present in the
59 environment mostly by **derived** from anthropogenic activities such as industrial
60 processes and urban traffic, and then transferred to the food chain (Pierron et al., 2014).
61 Numerous studies have shown that Cd stress leads to a wide variety of DNA damage

62 processes such as base-base mismatches, methylation, insertion/deletion loops, and
63 DNA chain crosslinking/breaks, which can result in genotoxicity or/and cytotoxicity to
64 cells (Filipic, 2012). Therefore, the study of the molecular mechanisms of Cd stress has
65 become a focus in ecotoxicology research (Wang et al., 2016).

66

67 Cell proliferation is a highly concerted and tightly regulated process controlled by the
68 cell cycle. This involves a highly conserved protein complex consisting of cyclin
69 dependent kinases (CDKs) and cyclins, which act as multiple regulating proteins (Jia et
70 al., 2016). Such CDK/cyclin complexes are required at cell cycle checkpoints, and
71 activation of cell cycle checkpoints is a major mechanism in preventing genetic
72 instability caused by threats originating from either exogenous environmental factors
73 (such as UV-B and heavy metals) or endogenous metabolic processes (such as
74 replication errors and metabolic byproducts) (Adachi et al., 2011; Cools and De Veylder,
75 2009; Hu et al., 2016). Schutter et al. (2007) demonstrated that Arabidopsis checkpoint
76 activation upon cessation of DNA replication/DNA damage is controlled by WEE1
77 kinase that operates in an ATM/ATR-dependent manner. To maintain genome integrity,
78 signaling cascades initiated by the phosphatidylinositol-3-OH kinase-like kinases ATM
79 and ATR control the activity of DNA repair complexes, halt cell cycle progression, and
80 in some cases, initiate cell death programs in plants and mammals (Hu et al., 2016; Jia
81 et al., 2016). In plants, the role of ATM/ATR-dependent signaling in the expression of
82 several DNA damage response and DNA repair genes, such as *GR1*, *MRE11*, *RAD51*
83 and *BRCA1*, has been demonstrated (Jia et al., 2016; Yoshiyama, 2016; Garcia et al.,
84 2003). Furthermore, in Arabidopsis *jing he sheng 1* (*jhs1*) and other seedlings, many
85 cell cycle-related genes such as *WEE1*, *CYCB1;1*, *CDKA;1*, *CDKB1;1*, *CYCD4;1*, *H3.1*,
86 and *CYCA2;1* were strongly induced upon DNA damage (i.e. endogenous DNA stress
87 and /or DNA double strand breaks (DSBs)-causing treatments), and the checkpoint
88 response is considered to be essential to inhibit transfer of damaged genetic
89 information to daughter cells, supporting genetic stability in the cells of organisms
90 (Cools and De Veylder, 2009; Culligan et al., 2006; Jia et al., 2016). Inhibition of
91 *CYCB1* and *CDKA* expression also occurred in response to Cd stresses in soybean

92 suspension culture cells, respectively (BursSENS et al. 2000; Sobkowiak and Deckert,
93 2004). Furthermore, Jiang et al. (2011) reported that UV-B-induced DNA damage
94 down-regulated expression of cell cycle related genes of *Histone H4* and *E2Fa* involved
95 in the G1/S transition in Arabidopsis root tips. However, little information is available
96 about the checkpoint response of cell cycle-related genes in Arabidopsis seedlings
97 under Cd stress (Pena et al., 2012).

98

99 DNA stress either changes or perturbs the duration of different stages of cell cycle in
100 plant cells, although the observed effects are dependent on plant species and tissue
101 tested as well as on the type and dose of the stress (Cools and De Veylder, 2009; Hu et
102 al., 2016; Jia et al., 2016). For example, endogenous replication stress caused by
103 mutation of replisome factor *E2F TARGET GENE 1 (ETG1)* induced a prolonged cell
104 cycle, accompanied with a high number of G2-phase cells in Arabidopsis (Cools and
105 De Veylder, 2009). G2-phase arrest also occurred in root cells of onion (*Allium cepa* L.)
106 reacting to X-ray-induced DSBs or hydroxyurea treatment (Pelayo et al., 2001; Carballo
107 et al., 2006). Recently, flow cytometry analysis indicated that the DNA damage
108 response may delay cell cycle progression and cause endoreduplication in Arabidopsis
109 *jhs1* mutant seedlings (Jia et al., 2016). In a variety of eukaryotic cells, signals induced
110 by Cd stress act at prereplication (G1/S) and/or premitosis (G2/M) checkpoints to inhibit
111 the cell cycle progression, and G2/M phase cells are more sensitive to the challenge of
112 several agents (Bakshi et al., 2008; Francis, 2011; Pena et al. 2012; Sobkowiak and
113 Deckert, 2004; Xie and Shaikh, 2006; Yang et al., 2004). However, little information is
114 known about cell cycle progression in response to Cd stress in Arabidopsis.

115

116 Therefore, the principal aims of this study were to (1) evaluate cell cycle progression in
117 response to Cd in Arabidopsis seedlings; (2) determine the expression levels of cell
118 cycle-related genes, including *CYCB1;1*, *CDKA;1*, *WEE1*, *E2Fa* and *Histone H4*, by
119 real-time, quantitative reverse transcription-PCR (qRT-PCR) analysis in Arabidopsis
120 under Cd stress; (3) explore potential associations between the cell cycle-related
121 indexes and expression of DNA damage marker genes in Arabidopsis under Cd stress.

122

123 **2. Materials and methods**

124 **2.1 Plant material, growth and treatment conditions**

125 *Arabidopsis thaliana* seeds (*Arabidopsis*, Columbia ecotype) were surface-sterilized in
126 bleach solution (1:10 dilution of hypochlorite) and ethanol mix (ethanol: water: bleach
127 7:2:1) at about 20 °C for 5 min, respectively. Seeds were rinsed in sterile distilled water
128 five times and imbibed in sterile-water for 2-4 days at 4 °C to obtain homogeneous
129 germination (Pedroza-Garcia et al., 2016). The seeds were then sown in sterile flasks
130 containing 150 mL of commercially available 0.5×Murashige and Skoog (MS) liquid
131 medium (Basalt Salt Mixure, Caisson, USA) with 0.5% (w/v) sucrose (pH 5.8), and
132 supplemented with Cd at a final concentration of 0 (the control), 0.125, 0.25, 1.0, and
133 2.5 mg·L⁻¹ in the form of CdCl₂·2H₂O of analytical grade with purity 99.5%, PR China.
134 Each flask with 20-30 plantlets was placed on a rotary shaker at about 50 rpm in an
135 incubator (12 h light of approximately 3000 lx and 12 h dark at 21 ± 0.5 °C) for 5 d. All
136 treatments and analyses were repeated in three independent replicates.

137

138 **2.2 RNA extraction and real-time, quantitative reverse transcription-PCR** 139 **(qRT-PCR) analysis**

140 For both the control and Cd treated plantlets, fresh root tip (about 0.5 cm) tissues were
141 collected after 5 d of growth as described above, and flash frozen in liquid nitrogen prior
142 to storage at -80 °C. Total RNA was isolated and purified using RNA isolation and clean
143 up kits (EZ-10 DNAaway RNA Mini-prep Kit, Sagon). First-strand cDNA was
144 synthesized from 2µg of total RNA using a PrimeScript™ 1st strand cDNA Synthesis Kit
145 (TaKaRa) according to the manufacturer's instructions. qRT-PCR analysis was done
146 using 20µL reaction mixtures containing 20 ng of template cDNA, 0.5µM of
147 corresponding forward and reverse primers and 10µL of 2×SYBR Mix (SYBR® Premix
148 Ex Taq™ II (Tli RNaseH Plus, TaKaRa). Reactions were run and analyzed on the
149 iCycler iQ (Bio-Rad) according to the manufacturer's instructions. The specificity of
150 amplification products was determined by melting curves. ACT2 was used for signals
151 normalization. IQ5 relative quantification software (Bio-Rad) automatically calculates

152 relative expression level of the selected genes with algorithms based on the $2^{-\Delta\Delta Ct}$
153 method (Livak and Schmittgen, 2001). Data were from triplicates and are
154 representative of at least three biological replicates. The sequence of primers used in
155 this study is provided in Supplementary Table S1.

156

157 **2.3 Flow Cytometry analysis of cell cycle progression in root tips of Arabidopsis**

158 Nuclei were extracted by chopping approximately 0.1g of fresh root tips (about 0.5 cm)
159 in ice cold Galbraiths Chopping buffer (45 mM MgCl₂; 30 mM sodium citrate; 20 mM
160 MOPS; 0.1% (w/v) TritonX-100; pH7.0) supplemented with 10 mM DTT in a Petri dish
161 with a razor blade (Hefner et al., 2006). After chopping, the tissue and buffer were
162 strained through 30 µm nylon mesh, and then 15 µg·mL⁻¹ RNase A were added and
163 incubated in a water bath of 37 °C for 30 min. The suspension was stained with 50 µ
164 g·mL⁻¹ propidium iodide (PI, Molecular Probes, Beyotime, PR China) at 4 °C for 30 min.
165 The control and Cd-treated samples were analyzed within 24 h by flow cytometry on a
166 FACSCalibur flow cytometer (Becton Dickinson, USA) equipped with a 488 nm laser.
167 Detector settings were determined empirically. Fluorescence intensity was analyzed in
168 the FL2 channel with no less than 10000 nuclei measured for each sample.

169

170 Quantitation was carried out by appropriately gating the raw data and comparing the
171 gated events for each peak and comparing that to the total number of gated nuclei.
172 Gates of Sup-Fig. 1 were determined empirically on nuclei isolated from the root tips of
173 the 5-day-old seedlings with FlowJo V10 software (BD Biosciences, San Jose, CA).
174 According to data in Sup-Fig. 1, analyses of cell cycle only calculated 2N, S and 4N (i.e.
175 100% in total for each treatment), and analyses of ploidy distribution only calculated 2N,
176 4N and 8N (i.e. 100% in total for each treatment).

177

178 **2.4. Statistical analysis**

179 SPSS for Windows (version 19.0) was used for the statistical evaluation of the results.
180 Values are expressed as mean ± standard deviation of the mean. Differences among
181 the control and treatments were analyzed by 1-way analysis of variance (ANOVA),

182 taking $P < 0.05$ as significant according to the least significant differences (LSDs) tests
183 corrected for the number of comparisons.

184

185 **3. Experimental results**

186 **3.1 Cd stress decreased root growth of Arabidopsis seedlings**

187 There were no statistically significant differences for fresh weight of shoots between the
188 control and Cd-treated seedlings (Table 1, $P < 0.05$) although shoots treated with 2.5
189 $\text{mg}\cdot\text{L}^{-1}$ of Cd indicated a slight decrease of fresh weight. Likewise, exposure to Cd of
190 0.125-2.5 $\text{mg}\cdot\text{L}^{-1}$ for 5 d had no obvious effect on the germination rate and chlorophyll
191 content of Arabidopsis seedlings compared to the control after 5 d of treatment (Table1,
192 $P < 0.05$). However, the differences between the root length of the control plantlets and
193 the plantlets treated with 1.0 and 2.5 $\text{mg}\cdot\text{L}^{-1}$ Cd were found to be statistically significant
194 ($P < 0.05$, Table 1). Indeed, in plantlets exposed to 0.125-2.5 $\text{mg}\cdot\text{L}^{-1}$ Cd, a significant
195 inverted U-shaped relationship was seen between the root length and Cd level (Table
196 1).

197

198 **3.2 Cd stress triggered cell cycle arrest in root tips of Arabidopsis**

199 To analyze cell cycle progression in Arabidopsis plantlets of 5-d-old seedlings under Cd
200 stress, the effects of Cd stress on cell cycle arrest were examined by flow cytometry. As
201 shown in Fig. 1, the proportion of cells with a ^2C nuclear content (G0/G1 phase) was
202 45.04% in the control plantlets whereas Cd stress significantly altered this proportion,
203 which was 50.33, 49.54, 39.71 and 29.86% under 0.125-2.5 $\text{mg}\cdot\text{L}^{-1}$ Cd stresses,
204 respectively. This alteration in the ^2C nuclear content was accompanied by changes in
205 the proportion of cells with a ^4C and ^8C nuclear content, which was 48.9, 47.0, 45.6,
206 57.5 and 74.6% in root tips of 0-2.5 $\text{mg}\cdot\text{L}^{-1}$ Cd-treatment, respectively (Table 2,
207 Sup-Fig. 1). There was no significant effect of Cd on cells in the S phase of the cell
208 cycle (Fig. 1). This result suggests that the G1/S phase of the cell cycle is significantly
209 delayed in the 0.125 $\text{mg}\cdot\text{L}^{-1}$ Cd-treated plantlets and that the G2/M phase of the cell
210 cycle is delayed in the 1.0-2.5 $\text{mg}\cdot\text{L}^{-1}$ Cd-treated plantlets, respectively.

211

212 **3.3 Cd stress induced the changes in expression of cell cycle-regulatory genes in**
213 **root tips of Arabidopsis seedlings**

214 The effect of Cd on the cell cycle-regulatory genes was further determined by
215 measuring the expression of marker genes for cell proliferation (*PCNA1* and *PCNA2*),
216 G1/S transition (*Histone H4* and *E2Fa*), and G2/M transition (*WEE1*, *CDKA;1* and
217 *CYCB1;1*) in root tips under Cd stress for 5 d by qRT-PCR analysis. An increase in the
218 gene expression of *PCNA1*, *CDKA;1*, and *WEE1* was observed in root tips exposed to
219 the lowest concentration ($0.125 \text{ mg}\cdot\text{L}^{-1}$) of Cd (Fig. 2), whereas a dose-dependent
220 decrease was seen in expression of these genes with $0.25\text{-}2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd, and in
221 *CYCB1;1*, *PCNA2*, *Histone H4* and *E2Fa* with $0.125\text{-}2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd, respectively (Fig.
222 2). Amongst all the Cd levels used for plantlet treatment, $0.125 \text{ mg}\cdot\text{L}^{-1}$ Cd caused a
223 increase of 1.1- to 1.4-fold in gene expression of *PCNA1* and *WEE1*, while $2.5 \text{ mg}\cdot\text{L}^{-1}$
224 Cd resulted in a maximum decrease of 1.4- to 2.5-fold in the expression of all of the cell
225 cycle-related genes (Fig. 2), respectively. These findings support the hypothesis that
226 Cd stress can modulate the expression of cell cycle regulatory genes involved in G1/S
227 and G2/M transitions in Arabidopsis root tips.

228

229 **3.4 Cd stress induced the changes in expression of DNA damage response**
230 **genes in root tips of Arabidopsis seedlings**

231 To examine DNA damage-response in the Cd-treated root tips of Arabidopsis seedlings,
232 we analyzed the expression of several marker genes for the DNA damage response
233 using qRT-PCR analysis. As shown in Fig. 2, the expression levels of *MRE11* and
234 **BRCA1** were increased approximately 1.1- to 1.8-fold in the $0.125\text{-}1.0 \text{ mg}\cdot\text{L}^{-1}$ Cd-treated
235 Arabidopsis, and decreased in the $2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd-treated Arabidopsis. However, a
236 concentration-dependent reduction in the expression of *GR1* with a minimum decrease
237 of 1.1-fold at $0.125 \text{ mg}\cdot\text{L}^{-1}$ Cd and a maximum decrease of 3.3-fold at $2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd
238 exposure was observed. Also, a dose-dependent decrease in the expression of DNA
239 mismatch repair genes, *MLH1*, *MSH2* and *MSH6*, by 1.2- to 4.2-fold was observed at
240 $0.125\text{-}2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd exposure, respectively, in the root tips of Arabidopsis seedlings in
241 comparison to the control. This result suggests that significant DNA damage occurred

242 in the root tips of Cd-treated plantlets for 5 d.

243

244 **4. Discussion**

245 Root tips are the most active region of plant roots for Cd influx, and Cd stress has
246 deleterious effects on plant growth and development (Filipic et al., 2012). Our results
247 indicated, however, that a significant reduction in root length appeared only in plants
248 exposed to Cd at 1.0 and 2.5 mg·L⁻¹ while a significant increase in root growth was
249 observed at lower (0.125 mg·L⁻¹) Cd after 5 d of treatment. However, exposure to Cd of
250 across the concentration range tested did not significantly affect fresh weight or
251 chlorophyll content of shoots (Table 1). A similar trend of a low dose of Cd stimulating
252 cell proliferation was reported in mouse testicular Leydig cells (Singh et al., 2009).
253 Upon salt stress, the root meristematic zone was decreased in Arabidopsis root tips
254 (West et al., 2004). Furthermore, in the aluminum (Al)-sensitive variety of maize, Al
255 exposure completely blocked the entrance of cells into the S-phase in the central part
256 of the root meristematic zone (250–800 μm from the apex) (Doncheva et al., 2005).
257 Therefore, this study suggests that Al stress first rapidly blocks cell cycle progression,
258 presumably to prevent the entrance into stages when the cells are particularly
259 vulnerable to DNA damage, and to allow the cellular defense system to be activated.

260

261 DNA can be impaired in a variety of manners under various stresses, originating from
262 either exogenous (such as UV-B and heavy metal stresses) or endogenous (such as
263 replication errors and ROS) sources. To maintain genome integrity, signaling cascades
264 initiated by ATM and ATR control the activity of DNA repair complexes, halt cell cycle
265 progression, and in some cases, initiate cell death programs in plants and mammals
266 (Hu et al., 2016; Jia et al., 2016). In plants, the role of ATM/ATR-dependent signaling in
267 the expression of several DNA damage response and DNA repair genes, such as *GR1*,
268 *MRE11*, *RAD51* and *BRCA1*, has been demonstrated (Jia et al., 2016; Yoshiyama,
269 2016; Garcia et al., 2003). However, we know very little about molecular players in
270 DNA damage response in Arabidopsis under Cd stress. Herein, we analyzed the
271 expression levels of key genes in DNA damage responses (Fig. 2). The expression of

272 *MLH1*, *MSH2* and *MSH6* was significantly reduced in the Cd-treated Arabidopsis in
273 comparison to the control. These genes play important roles in the recognition and
274 correction of damaged DNA bases, pyrimidine dimers and mismatches such as
275 mispaired or unpaired bases, in the activation of cell cycle checkpoints, and in
276 maintaining the stability of genomic DNA and fidelity of DNA replication etc (Lario et al.,
277 2011); the decrease in their expression under Cd stress is likely therefore to result in
278 DNA damage in these plantlets. The expression of *BRCA1*, which functions in genome
279 surveillance and DNA damage repair (Jia et al., 2016), was enhanced more than 1.1- to
280 1.6-fold in the 0.125-1.0 mg·L⁻¹-Cd-treated Arabidopsis and decreased in the 2.5
281 mg·L⁻¹-Cd-treated Arabidopsis, respectively (Fig. 2), suggesting that DNA damage
282 appears in these seedlings exposed to Cd stress. Similarly, the *MRE11* nuclease,
283 which is involved in DSB end processing (Roth et al., 2012), was significantly induced
284 more than 1.2- to 1.8-fold in the 0.125-1.0 mg·L⁻¹-Cd-treated Arabidopsis, and
285 decreased in the 2.5 mg·L⁻¹-Cd-treated seedlings, respectively. Also, the *GR1* nuclease,
286 which are involved in DSB end processing (Roth et al., 2012), was significantly
287 decreased in the seedlings of 0.125-0.25 mg·L⁻¹ Cd-treatment, respectively (Fig.2). The
288 above results suggest that at low levels of Cd, the increased expression of the genes
289 involved in DNA damage repair likely decreases the amount of damaged DNA in the
290 Cd-treated cells perhaps enhancing cell proliferation and hence root extension.
291 However at higher Cd concentrations the DNA damage is extensive, resulting in
292 decreased expression of these genes. This would indicate a dysfunctional repair
293 system further increasing the DNA damage (Sup-Fig. 2; Wang et al., 2016).

294

295 The mechanisms underlying a DNA damage response–dependent cell cycle arrest
296 have been well characterized in mammals, and relatively little has been known in plant
297 cells (Adachi et al., 2011; Cools and De Veylder, 2009; Filipic, 2012; Hu et al., 2016). In
298 the current experiment, the results indicate that Cd stress does affect expression
299 patterns of cell cycle regulatory genes involved in G1/S transition and G2/M transition in
300 root tips of Arabidopsis seedlings (Fig. 2). This suggests that Cd may have an adverse
301 effect on the regulatory process of the checkpoints of G1/S and G2/M checkpoint

302 transitions in the Arabidopsis seedlings. Although the accurate timing and role of
303 various gene products at specific stages of the cell cycle has not been clearly
304 elucidated under Cd stress, we show here that the expression of several genes
305 involved in cell cycle regulation is affected differentially by the Cd exposure levels. For
306 example, expression of **three** genes involved in the G2/M transition (i.e. *WEE1*,
307 *CDKA;1* and *PCNA1*) was significantly induced by exposure to $0.125 \text{ m}\cdot\text{L}^{-1}$ of Cd for 5
308 d, and therein *WEE1* and *CDKA;1* were **a critical regulatory factor** and the composition
309 of MPF (Maturation Promoting Factor) engaged in G2/M transition, respectively
310 (O'Connell et al., 1997). However, substantially down-regulated expression occurred at
311 0.25 , 1.0 and $2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd for the above three genes and at 0.125 - $2.5 \text{ mg}\cdot\text{L}^{-1}$ Cd for
312 four genes (*CYCB1;1*, *Histone H4*, *PCNA2* and *E2Fa* which is crucial for G1/S or G2/M
313 transition) tested, respectively (Fig. 2). However, a increase of 3.1- to 4.7-fold in gene
314 expression of *CYCB1;1* and *WEE1* occurred in Arabidopsis plantlets exposed to
315 0.125 - $0.25 \text{ mg}\cdot\text{L}^{-1}$ Cd for 24 h **respectively, when** Arabidopsis grew under the untreated
316 control condition for about 5 d at 21°C after germination (data not given). Similar trend
317 was reported on mouse testicular Leydig cells, soybean suspension-cultured cells,
318 parsley, maize, wheat and Arabidopsis species under stresses of fungal elicitor, low
319 temperature, UV irradiation, salt and Cd, respectively (Pena et al., 2012; Singh et al.,
320 2009; Sobkowiak et al., 2003; Rymen et al., 2007; Xie and Shaikh, 2006). Alternatively,
321 Pena et al. (2012) reported that Cd stress down-regulated expression of *PCNA* in
322 wheat root apical meristems, which is cell cycle marker gene related to G1/S transition
323 through the E2F/retinoblastoma-related (RBR) pathway. Moreover, expression of
324 *PCNA* gene in rice seedlings was induced by exposure to **a DNA-damage agent**, such
325 as UV of $25 \text{ J}\cdot\text{m}^{-2}$ and H_2O_2 of 1 mM treatment, indicating that the biomarker responses
326 could be used to differentiate stress effect (Yamamoto et al., 2005). Therefore, modified
327 expression of the cell cycle regulatory genes involved in **G1/S transition** and G2/M
328 transition probably supports the assumption that Cd stress would be responsible for the
329 **decrease/decrease** in cell proliferation through G1/S or/and G2/M checkpoint arrest in
330 Arabidopsis root tips in the current research.

331

332 Cd, as a redox inactive metal, changed expression of DNA damage response and cell
333 cycle regulatory genes in the root tips of Arabidopsis seedlings by qRT-PCR analysis,
334 and dose-dependent manners between Cd levels applied and expression of cell cycle
335 regulatory genes are notably reduced or inverted U-shaped curves with the maximum
336 effect at 0.125-1.0 mg·L⁻¹ Cd, respectively, (Fig. 2), which is in agreement with the
337 findings of previous reports (Singh et al., 2009; Liu et al., 2009; De Schutter et al.,
338 2007). The major mechanistic explanations for the induced expression of the above
339 genes observed by low levels of Cd are a likely modulation of cellular signal
340 transduction pathways by activation of transcription factors or/and modification of
341 protein phosphorylation status as a result of the interplay among ATM, ATR, SOG1,
342 WEE1 kinases, CDC25 phosphatases and CDKA;1 (Cools and De Veylder, 2009; Hu
343 et al., 2016). Emerging study has demonstrated that upon different types of DNA stress
344 from the Arabidopsis plants, the transcriptional activation of DNA repair and cell cycle
345 checkpoint genes totally depends on ATM and/or ATR, suggesting that ATM and ATR
346 could play a pivotal role in the DNA-damage checkpoint response in plants (De
347 Schutter et al., 2007; Cools and De Veylder, 2009; Hu et al., 2016). Similarly, mutations
348 in ATM or ATR render organisms hypersensitive to DNA damage-inflicting agents in
349 plants, and the ATM mutants show growth defects when treated with γ-rays or methyl
350 methanesulfonate (MMS), causing DSBs (Cools and De Veylder, 2009). Also, low level
351 of Cd can interfere with antioxidant defense systems and stimulate the production of
352 highly reactive free radicals in cells (Filipic, 2012). Thus, these reactive free radicals in
353 cells may act as signaling molecules and induce expression of cell cycle regulatory
354 genes in the Arabidopsis seedlings in this experiment (Fig. 2). Cools and De Veylder
355 (2009) demonstrated that the unique behavior of increased CYCB1;1 expression hints
356 at a specific function for this particular cyclin in DNA-stress response, but the role is
357 unknown, which seems that increased CYCB 1;1 levels maintain the stressed cell's
358 competence for cell division. Alternatively, Cd has a high affinity to cysteine in three
359 dimensional protein structures and can promote specific binding of Cd to the above
360 protein components (Filipic, 2012), which can inhibit expression of DNA repair and cell
361 cycle regulatory genes tested under Cd stress (Fig. 2). Decreases in mRNA stability

362 and increased mRNA turnover rates are other possible explanations for the observed
363 changes in expression for DNA repair and cell cycle regulatory genes (Fig. 2). Since
364 the ubiquitin-proteasome system (UPS) is particularly important for the turnover of
365 many cyclins-like critical proteins participating in cell proliferation process, and cyclin D
366 and CDKA proteins conjugated with highly conserved 76-aminoacid protein ubiquitin
367 (Ub) were specifically decreased in wheat root tips under Cd stress (Pena et al., 2012),
368 reduction of the cell cycle regulatory genes could then represent a protective response
369 to Cd stress in this research (Fig. 2). All the Cd effects mentioned above undoubtedly
370 would severely affect modifications in expression of the genes observed of Arabidopsis
371 seedlings exposed to Cd in the current study.

372

373 It is well known that when cells suffer different kinds of DNA stresses, G1/S and G2/M
374 checkpoints can be activated **that** transiently inhibit cell proliferation so that DNA
375 lesions can be repaired before the cell cycle continues, respectively, and the above
376 effect could be performed via SOG1 transcription factor activated by ATM/ATR in
377 plants (Cools and De Veylder, 2009; Fulcher and Sablowski, 2009; Hu et al., 2016;
378 Furukawa et al., 2010; Yoshiyama, 2016). In the current study, the reduced growth of
379 the root tips in the 1.0-2.5 mg·L⁻¹ -Cd-treated seedlings suggests that a cell cycle delay
380 was triggered (Tables 1 and 2, Fig. 1). Cd stress markedly delayed progression of G1/S
381 transition at 0.125 mg·L⁻¹ Cd and of G2/M transition at 1.0-2.5 mg·L⁻¹ Cd in Arabidopsis
382 root tips, concomitantly with enhanced DNA damage levels in Arabidopsis root tip cells
383 (Figs. 1 and 2; Sup-Fig. 2), which illustrates that DNA damage checkpoints occurred in
384 the Cd-treated plantlets. In immortalized human normal prostate epithelial cell line
385 (NPrEC), Bakshi et al. (2008) observed the G1/S arrest after 8 h of exposure to Cd,
386 whereas 32 h exposure caused the G2/M arrest. Jiang et al. (2011) showed that
387 UV-B-induced DNA damage delayed G1/S transition in Arabidopsis root tips at least
388 partially through changes in the regulation of the expression of cell cycle-related genes
389 *Histone H4* and *E2Fa*. It was reported that signals induced by Cd stress act at G1/S
390 or/and G2/M checkpoints to inhibit the cell cycle progression in a variety of eukaryotic
391 cells (Choi et al., 2011; Pena et al. 2012; Sobkowiak and Deckert, 2004; Xie and Shaikh,

2006; Yang et al., 2004). Moreover, the aberrant expression level of several genes related to G1/ S transition and G2/M transition occurred in the Cd-treated plantlets (Fig.2), and DNA damage can affect cell cycle progression partially through changes in the mediation of the expression of cell cycle-related genes (Jia et al., 2016). Thus, all these data support the notion that the DNA damage response sensed by *BRCA1* and MMR genes can delay G1/S transition by inhibiting E2F transcription factor which further suppresses expression of the above genes (i.e. *MSH6*, *PCNA1* and *PCNA2*), leading to **delay** G2/M transition during the cell cycle progression in Arabidopsis under Cd stress (Lario et al., 2011; Pena et al., 2012). In addition, 0.125 mg·L⁻¹ Cd-induced DNA damage induced the marked G1/S arrest but **shorted** G2/M phase, leading to accelerating growth in root tips, while 1.0-2.5 mg·L⁻¹ Cd-induced DNA damage caused the notable G2/M arrest in root tips, causing **reducing** growth in root tips (Tables.1-2, Figs.1-2, Sup-Fig. 1). As cell cycle progression is directly related to the cell division, proliferation, growth and development (Gutierrez et al. 2002), our results suggests that Cd-induced G1/S or/and G2/M arrest can be a protective mechanism that alleviates/prevents cells with damaged DNA from dividing and may provide more explanation for the reduction in crop growth and productivity under Cd stress. Also, MMR genes with their most sensitivity and lability could be **a** brilliant biomarker for Cd stress.

411

412 **Conclusions**

413 The present report defines modifications in cell cycle progression in correlation with the alteration of expression of cell cycle regulatory genes and DNA damage response genes measured in root tips of Arabidopsis seedlings exposed to Cd of 0.125-2.5 mg·L⁻¹ for 5 d. We observed the prominently inverted U-shaped dose-response effects of Cd stress on gene expression of *BRCA1*, *MRE11*, *WEE1*, *CDKA;1* and *PCNA1* in root tips of Arabidopsis seedlings at 0.125-2.5 mg·L⁻¹ Cd. Also, substantially decreased expression of genes was observed for *CYCB1;1*, *Histone H4*, *E2Fa*, *PCNA2*, *GR1*, *MSH2*, *MSH6* and *MLH1* in root tips exposed to 0.125-2.5 mg·L⁻¹ Cd for 5 d, respectively. Furthermore, Cd-induced DNA damage results in the significant delay of

422 G1/S **transition** and G2/M transition at 0.125 and 1.0-2.5 mg·L⁻¹ Cd in Arabidopsis root
423 tips, respectively. Cd-induced G1/S or/and G2/M arrest may be a protective mechanism
424 that prevents cells with damaged DNA from dividing and may explain the plant growth
425 inhibition under Cd stress.

426

427 **Acknowledgment**

428 This research was financially sponsored by National Natural Science Foundation of
429 China (NSFC, 21677151, 41673132, and 41472237), and The national key research
430 and development plan (2016YFD0800305).

431

432 **References**

433

434 Adachi, S., Minamisawa, K., Okushima, Y., Inagaki, S., Yoshiyama, K., Kondou, Y., Kaminuma, E.,
435 Kawashima, M., Toyoda, T., Matsui, M., Kurihara, D., Matsunaga, S., Umeda, M., 2011.
436 Programmed induction of endoreduplication by DNA double-strand breaks in Arabidopsis. *Plant*
437 *Biol.*108, 10004-10009.

438 Bakshi, S., Zhang, X., Godoy-Tundidor, S., Cheng, R. Y., Sartor, M. A., Medvedovic, M., Ho, S. M.,
439 2008. Transcriptome analyses in normal prostate epithelial cells exposed to low-dose cadmium:
440 oncogenic and immunomodulations involving the action of tumor necrosis factor. *Environ. Health*
441 *Perspect.*116, 769-776.

442 Beemster, G. T. S., De Veylder, L., Vercruyssen, S., West, G., Rombaut, D., Van Hummelen, P.,
443 Galichet, A., Grissem, W., Inze, D., Vuylsteke, M., 2005. Genome-wide analysis of gene
444 expression profiles associated with cell cycle transitions in growing organs of Arabidopsis. *Plant*
445 *Physiol.* 138, 734–743.

446 Burssens S., Himanen K., van de Cotte B., Beeckman T., Van Montagu M., Inzé D., Verbruggen N.,
447 2000. Expression of cell cycle regulatory genes and morphological alterations in response to
448 salt stress in Arabidopsis thaliana. *Planta.* 211, 632-640.

449 Carballo, J. A., Pincheira, J., De La Torre, C., 2006. The G2 checkpoint activated by DNA damage
450 does not prevent genome instability in plant cells. *Biol. Res.*39, 331-340.

451 Choi, Y. J., Yin, H. Q., Suh, H. R., Lee, Y. J., Park, S. R., Lee, B. H., 2011. Involvement of E2F1

452 transcriptional activity in cadmium-induced cell-cycle arrest at G1 in human lung fibroblasts.
453 Environ. Mol. Mutagen. 52, 145-152.

454 Cools, T., De Veylder, L., 2009. DNA stress checkpoint control and plant development. Curr. Opin.
455 Plant Biol. 12, 23-28.

456 Culligan K. M. , Robertson C. E., Foreman J., Doerner P., Britt, A. B., 2006. ATR and ATM play both
457 distinct and additive roles in response to ionizing radiation. Plant J. 48, 947-961.

458 De Schutter, K., Joubes, J., Cools, T., Verkest, A., Corellou, F., Babychuk, E., Van Der Schueren, E.,
459 Beeckman, T., Kushnir, S., Inze, D., De Veylder, L., 2007. Arabidopsis WEE1 kinase controls cell
460 cycle arrest in response to activation of the DNA integrity checkpoint. Plant cell. 19, 211-225.

461 Doncheva, S., Amenos, M., Poschenrieder, C., Barcelo, J., 2005. Root cell patterning: a primary
462 target for aluminium toxicity in maize. J. Exp.Bot. 56, 1213-1220.

463 Filipic, M., 2012. Mechanisms of cadmium induced genomic instability. Mutat. Res. 733, 69-77.

464 Francis, D., 2011. A commentary on the G2/M transition of the plant cell cycle. Ann. Bot. 107,
465 1065-1070.

466 Hefner, E., Huefner, N., Britt, A. B., 2006. Tissue-specific regulation of cell-cycle responses to DNA
467 damage in Arabidopsis seedlings. DNA Repair. 5, 102-110.

468 Hsieh, Y. H., Lee, C. H., Chen, H. Y., Hsieh, S. C., Lin, C. L., Tsai, J. P., 2015. Induction of cell cycle
469 arrest, DNA damage, and apoptosis by nimbolide in human renal cell carcinoma cells. Tumor
470 Biol. 36, 7539-7547.

471 Hu, Z. B., Cools, T., De Veylder, L., 2016. Mechanisms used by plants to cope with DNA damage.
472 Annu. Rev. Plant Biol. 67, 439-462.

473 Jia, N., Liu, X. M., Gao, H. B., 2016. A DNA2 homolog is required for DNA damage repair, cell cycle
474 regulation, and meristem maintenance in plants. Plant physiol. 171, 318-333.

475 Jiang, L., Wang, Y., Bjorn, L. O., Li, S.S., 2011. Arabidopsis radical-induced cell death1 is involved in
476 UV-B signaling. Photochem. Photobiol. Sci. 8:838-846.

477 Jin, Y. H., Clark, A. B., Slebos, R. J. C., Al-Refai, H., Taylor, J. A., Kunkel, T. A., Resnick, M. A.,
478 Gordenin, D. A., 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat. Genet.
479 34, 326-329.

480 Liu, W., Zhou, Q. X., Li, P. J., Gao, H. R., Han, Y. P., Li, X. J., Yang, Y. S., Li, Y. Z., 2009. DNA
481 mismatch repair related gene expression as potential biomarkers to assess cadmium exposure

482 in Arabidopsis seedlings. *J. Hazard Mater.* 167, 1007-1013.

483 Livak, K. J., Schmittgen, T. D., 2001. Analysis of relative gene expression data using real-time
484 quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. *Methods.* 25, 402-408.

485 Logemann, E., Wu, S. C., Schröder, J., Schmelzer, E., Somssich, I. E., Hahlbrock, K., 1995. Gene
486 activation by UV light, fungal elicitor or fungal infection in *Petroselinum crispum* is correlated with
487 repression of cell cycle-related genes. *Plant J.* 8, 865-876.

488 O'Connell, M. J., Raleigh, J. M., Verkade, H. M., Nurse, P., 1997. Chk1 is a wee1 kinase in the G₂
489 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. *EMBO J.* 16, 545-554.

490 Pedroza-Garcia, J. A., Domenichini, S., Mazubert, C., Bourge, M., White, C., Hudik, E., Bounon, R.,
491 Tariq, Z., Delannoy, E., Del Olmo, I., Pineiro, M., Jarillo, J. A., Bergounioux, C., Benhamed, M.,
492 Raynaud, C., 2016. Role of the polymerase sub-unit DPB2 in DNA replication, cell cycle
493 regulation and DNA damage response in Arabidopsis. *Nucleic Acids Res.* 44, 7251-7266.

494 Pelayo, H. R., Lastres, P., De la Torre, C., 2001. Replication and G₂ checkpoints: their response to
495 caffeine. *Planta.* 212, 444-453.

496 Pena, L. B., Barcia, R. A., Azpilicueta, C. E., Mendez, A. A., Gallego, S. M., 2012. Oxidative post
497 translational modifications of proteins related to cell cycle are involved in cadmium toxicity in
498 wheat seedlings. *Plant Sci.* 196, 1-7.

499 Pineros, M. A., Shaff, J. E., Kochian, L. V., 1998. Development, characterization, and application of
500 a cadmium-selective microelectrode for the measurement of cadmium fluxes in roots of *Thlaspi*
501 species and wheat. *Plant Physiol.* 116, 1393-1401.

502 Ramirez-Parra, E., Gutierrez, C., 2007. E2F regulates FASCIATA1, a chromatin assembly gene
503 whose loss switches on the endocycle and activates gene expression by changing the
504 epigenetic status. *Plant Physiol.* 144, 105-120.

505 Rymen, B., Fiorani, F., Kartal, F., Vandepoele, K., Inze, D., Beemster, G. T., 2007. Cold nights impair
506 leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle
507 genes. *Plant Physiol.* 143, 1429-1438.

508 Singh, K. P., Kumari, R., Pevey, C., Jackson, D., DuMond, J. W., 2009. Long duration exposure to
509 cadmium leads to increased cell survival, decreased DNA repair capacity, and genomic
510 instability in mouse testicular Leydig cells. *Cancer Lett.* 279, 84-92.

511 Sobkowiak, R., Deckert, J., 2003. Cadmium-induced changes in growth and cell cycle gene

512 expression in suspension-culture cells of soybean. *Plant Physiol. Biochem.* 41, 767-772.

513 Sobkowiak, R., Deckert, J., 2004. The effect of cadmium on cell cycle control in suspension culture
514 cells of soybean. *Acta Physiol. Plant.* 26, 335-344.

515 Sugimoto-Shirasu, K., Roberts, K., 2003. "Big it up": endoreduplication and cell-size control in plants.
516 *Curr. Opin. Plant Bio.* 6, 544-553.

517 West, G., Inze, D., T.S. Beemster, G., 2004. Cell cycle modulation in the response of the primary root
518 of Arabidopsis to salt stress. *Plant physiol.* 135, 1050-1058.

519 Xie, J. X., Shaikh, Z. A., 2006. Cadmium induces cell cycle arrest in rat kidney epithelial cells in
520 G₂/M phase. *Toxicology.* 224, 56-65.

521 Yamamoto, T., Mori, Y., Ishibashi, T., Uchiyama, Y., Ueda, T., Ando, T., Hashimoto, J., Kimura, S.,
522 Sakaguchi, K., 2005. Interaction between proliferating cell nuclear antigen (PCNA) and a DnaJ
523 induced by DNA damage. *J.plant Res.* 118, 91-97.

524 Yamane, K., Schupp, J. E., Kinsella, T. J., 2007. BRCA1 Activates a G₂-M cell cycle checkpoint
525 following 6-Thioguanine-Induced DNA mismatch damage. *Cancer Res.* 67, 6286-6292.

526 Yang, P. M., Chiu, S. J., Lin, K. A., Lin, L. Y., 2004. Effect of cadmium on cell cycle progression in
527 chinese hamster ovary cells. *Chem. Biol. Interact.* 149, 125-136.

528 Yoshiyama, K. O., 2015. SOG1: a master regulator of the DNA damage response in plants. *Genes*
529 *Genet Syst.* 90, 209-216.

530 Zhou, B. B. S., Elledge, S. J., 2000. The DNA damage response:putting checkpoints in perspective.
531 *Nature.* 408, 433-439.

532

Table 1. Effect of Cd stress on germination, total chlorophyll level, fresh weight and root growth of Arabidopsis seedlings for 5 d.

Cd level /mg·L ⁻¹	Germination percentage/ %	Total chlorophyll / $\mu\text{g}\cdot\text{g}^{-1}$ FW	Fresh weight /mg·shoot ⁻¹	Root growth	
				Root length/ cm	Inhibitory rate/ %
0	96.1±2.1	328.4±23.2	10.03±0.85	1.29±0.03	0
0.125	96.2±1.3	330.8±26.1	11.56±1.72	1.48±0.02a	-14.73
0.25	95.6±1.5	326.7±24.5	11.01±1.90	1.35±0.03	-0.51
1.0	95.2±2.7	331.3±21.9	10.04±1.26	1.07±0.04a	17.05
2.5	94.6±3.2	312.5±20.6	9.03±0.51a	0.76±0.01a	41.09

^aSignificantly different from the control ($P < 0.05$). Data are means \pm SE (n = 3).

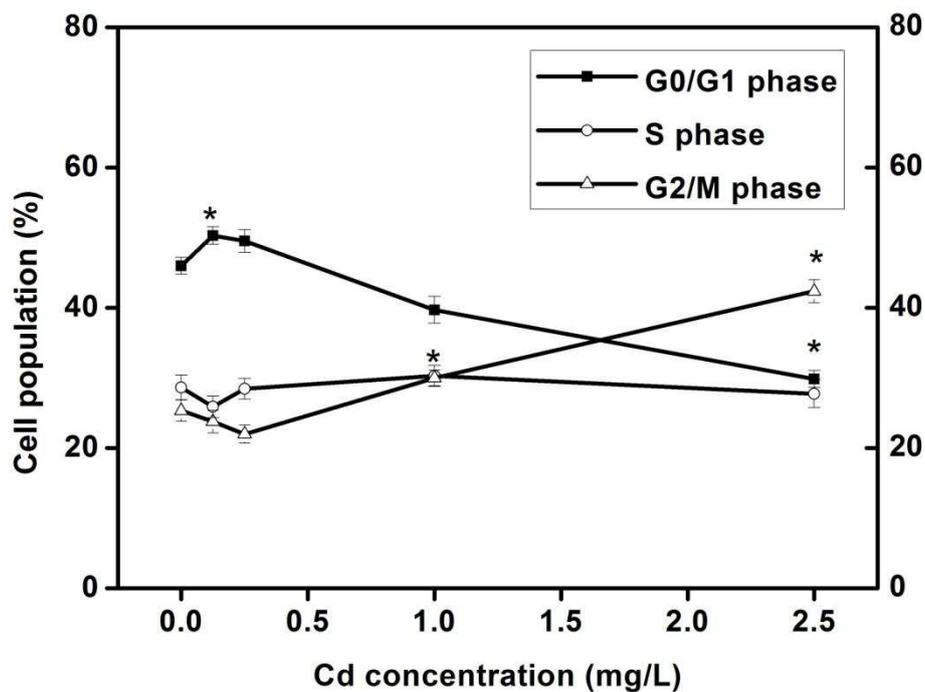


Fig.1. Effect of Cd on the cell cycle in root tips of Arabidopsis for 5 d. The percent distribution of cells in G0/G1, S, and G2/M phases was calculated and compared with the control. Each point represents the mean \pm S.D. of three independent experiments. *Significantly different from the control cells ($P < 0.05$), the same below.

Table 2. Effects of Cd stress on the distribution of DNA content in root tips of Arabidopsis for 5 d.

DNA content(%)	Cd concentration(mg/L)				
	0	0.125	0.25	1.0	2.5
2C	51.1	53.0	54.4	42.5	25.4
4C	28.2	25.1	24.1	32.1	35.9
8C	20.7	21.9	21.5	25.4	38.7*

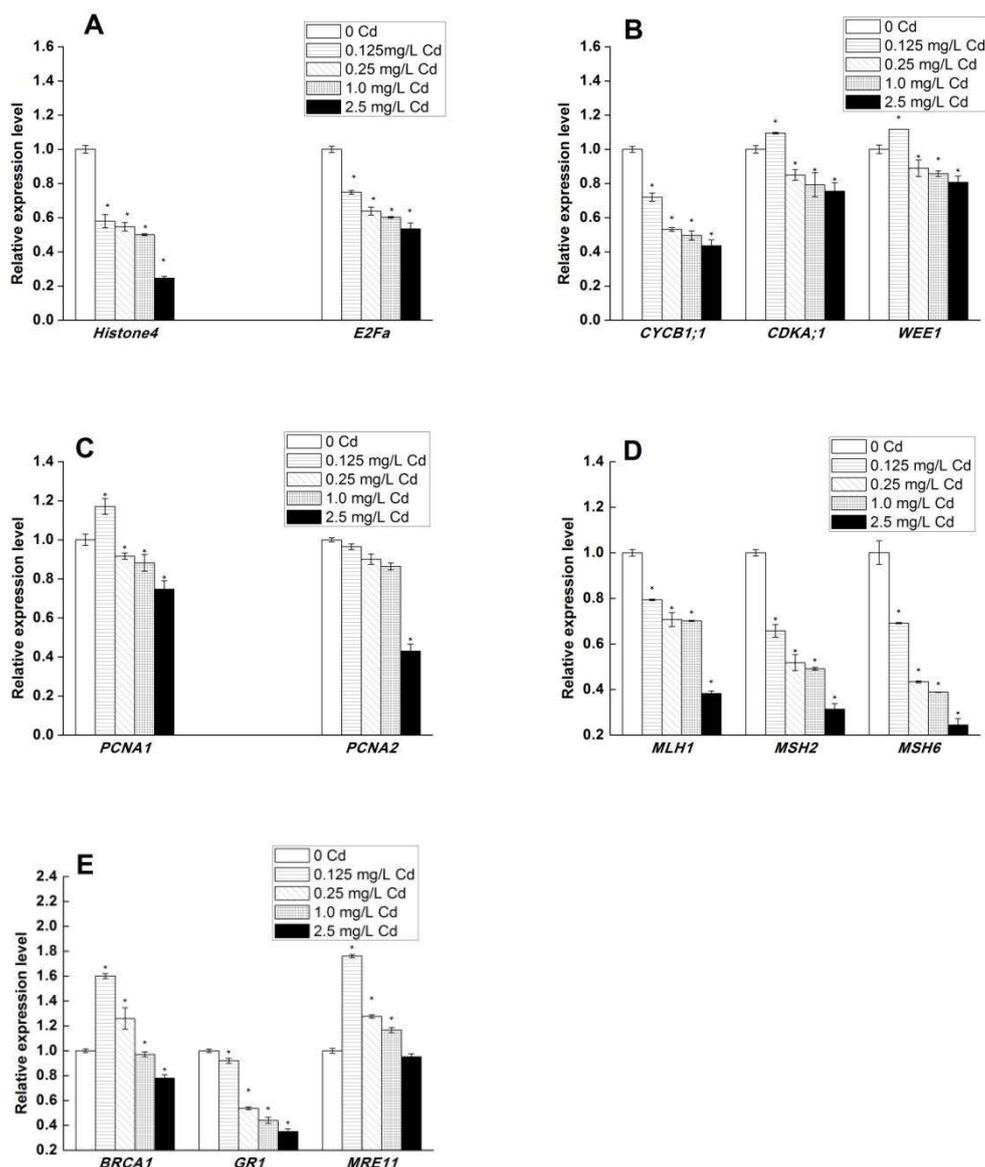


Fig.2. Effects of Cd stress on gene expression in root tips of Arabidopsis for 5 d.

(A) G1/S marker genes *Histone H4* and *E2Fa*; (B) G2/M marker genes *CYCB1;1*, *CDKA;1* and *WEE1*; (C) Cell proliferation marker genes *PCNA1* and *PCNA2*; (D) DNA mismatch repair genes *MLH1*, *MSH2* and *MSH6*; (E) DNA damage repair genes *BRCA1*, *GR1* and *MRE11*. Data are shown as mean \pm SD by qRT-PCR. Data presented are average of three replicates. House-keeping gene *AtACT2* was used as an internal control.

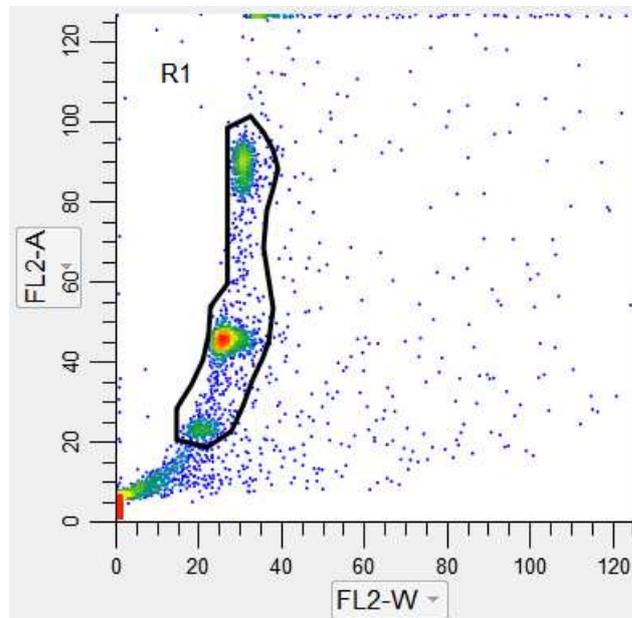
Supplementary material**Sup--Table 1.** Primer sequences used

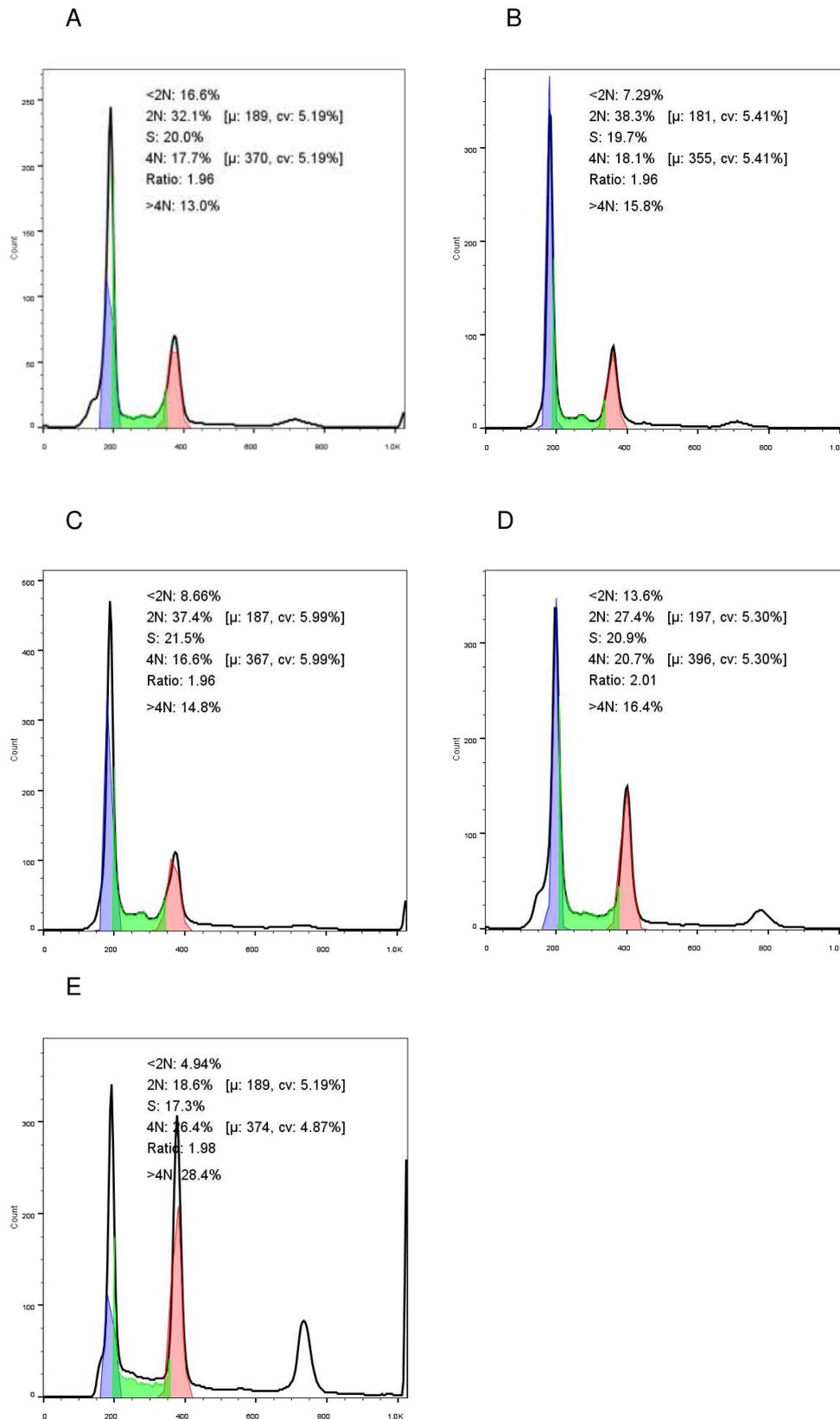
Gene name	Forward primer (5' —3')	Reverse primer (5' —3')	PCR product size (bp)
<i>ACT2</i> ^[a]	TCGTGGATTCCAGCAGCTTCC	CCGATGGGCAAGTCATCACG	100
<i>MLH1</i> ^[b]	GTAGTAAGGTCTTCTGCAAGGCA	TGCCATTCCAACATATGTGC	147
<i>MSH2</i> ^[b]	TCTGACTAGGCGAGTTCTT	CACCTCTCCAGGGAATCA	162
<i>MSH6</i> ^[b]	ATTAGTTAGAAAGGGCTATCGGG	AACAACCTGCACATACTTCGC	127
<i>Histone4</i> ^[a]	GATTCGTCGTCTTGCTCGTAG	CAGTCACCGTCTTCCTCCTC	149
<i>E2Fa</i> ^[a]	ACCATCCACCGTCATCTC	GCTCCTGTCGTTATTACTG	158
<i>CYCB1;1</i> ^[c]	CTCAAATCCCACGCTTCTTGTGG	CACGTCTACTACCTTTGGTTTCCC	110
<i>CDKA;1</i> ^[c]	CCTGTCAGGACATTTACTCATGAG	GCTTTTGGCTGATCATCTCAGC	139
<i>WEE1</i> ^[d]	TGGTGCTGGACATTTTCAGTCGG	CAAGAGCTTGCACCTCCATCATAG	137
<i>PCNA1</i> ^[b]	GTGACACAGTTGTGATCTCTG	ATCACAATTGCATCTTCCGG	127
<i>PCNA2</i> ^[b]	GATGAAGCTGATGGATATCGAC	GAGATCACAACCTGTGTCACC	138
<i>GR1</i> ^[c]	CAGCATGAGAAATCAGCAATCTCG	GGTGAGATGGAAGTGATAGGTGTC	161
<i>BRCA1</i> ^[c]	GTAACCATGTATTTTGAATGCGTG	GTGACGGATTATTCTGGCTAACG	192
<i>MRE11</i> ^[c]	GTGATACACTTCGAGTACTTGTTGC	CTGACTACTTGAAACTGCACTGG	256

[a] Jiang et al. 2011; [b] Liu et al. 2009; [c] Jia et al., 2016; [d] Cools and De Veylder, 2009.

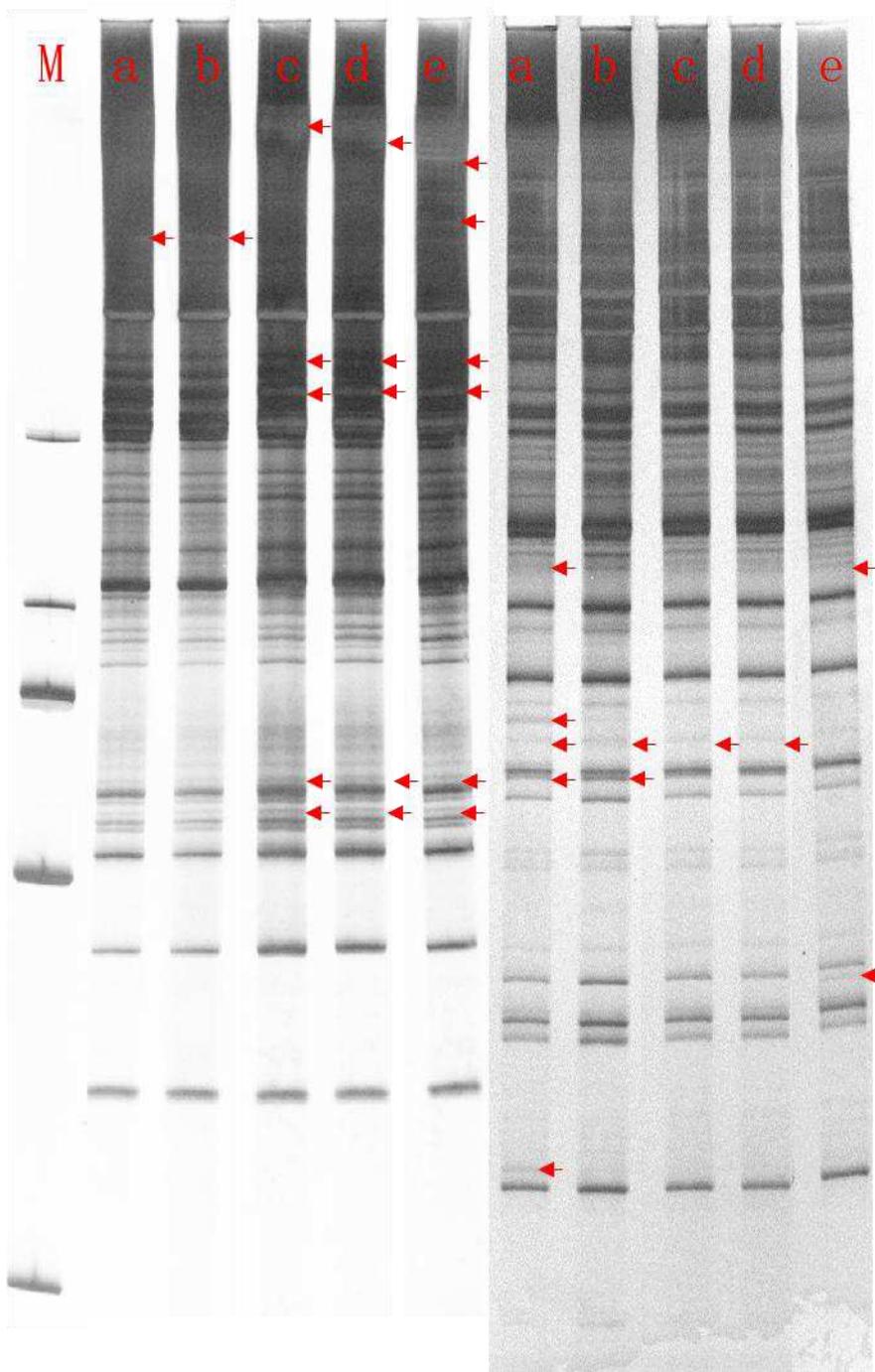
The same as the reference.

A'





Sup--Fig. 1 Effects of different concentrations of Cd on DNA distribution in Arabidopsis seedling root tip cells determined by flow cytometry analysis. Fluorescence-2 area (FL2-A) is a measure of integrated cell fluorescence signal that represents the DNA content. Data represent results from three replicates. A-E represents 0, 0.125-2.5 mg/L Cd, A', PI fluorescence signal, respectively.



Sup--Fig. 2 RAPD fingerprints of *Arabidopsis* seedlings exposed to 0~2.5 mg·L⁻¹ Cd for 5 d. a-e represents 0, 0.125-2.5 mg/L Cd, respectively.