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PAN POLSKA AKADEMIA NAUK ODDZIAŁ W KRAKOWIE

ARABIDOPSIS CYCLIN-DEPENDENT KINASE GENE *CDKG;2* IS INVOLVED IN ORGANOGENIC RESPONSES INDUCED IN VITRO

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The *Arabidopsis CDKG;2* gene encodes a putative cyclin-dependent Ser/Thr protein kinase of unknown biological function. This gene shows structural similarity to animal and human cyclin-dependent (PITSLRE) kinases. This study used the homozygous knockout *cdkg;2* mutant based on T-DNA insertional line SALK_090262 to study the effect of mutation of the *CDKG;2* gene on explant response and in vitro plant regeneration. For callus induction and proliferation, hypocotyls and cotyledons of 3-day-old seedlings of *cdkg;2* and *A. thaliana* ecotype Col-0 were cultured on solid MS medium supplemented with 2,4-D (2 mg l^{-1}). Organogenesis was induced after callus transfer on MS + TDZ (0.5 mg l^{-1}). The initiation time of callus and shoot induction differed between the mutant and control cultures. Shoot regeneration after callus transfer on MS + TDZ was delayed in *cdkg;2* (31 days versus 7 days in Col-0). Shoots formed on callus derived from Col-0 hypocotyls but not on cotyledon-derived callus; in *cdkg;2*, shoots developed on both callus types. Mutant shoots did not form roots, regenerants were dwarfed, and inflorescences had small bud-like flowers with a reduced corolla and generative organs. Abnormalities observed during *cdkg;2* organogenesis suggest a role of CDKG;2 as a regulator of adventitious root initiation.

Key words: *Arabidopsis thaliana* cyclin-dependent kinases, *CDKG;2* gene, callus formation, organogenesis.

INTRODUCTION

Cell division is a key process which determines plant growth and morphogenesis. Reversible phosphorylation by highly specific serine-threonine protein kinases and phosphatases, modulated by the binding of additional regulatory and scaffolding proteins, is a mechanism crucial for control of cell cycle progression and is conserved in all eukaryotic organisms (Dewitte and Murray, 2003). In particular, cyclin-dependent kinases (CDK) that require complexation with a cyclin subunit for their activity play a pivotal role in phosphorylation of target proteins, which are indispensable for the orderly progression of cells through the phases of the cell cycle (Inze and de Veylder, 2006). Over 150 putative CDK-encoding genes have been identified in numerous plant species. Based on motifs conserved within the sequences of cyclin-binding domains, the broad family of putative CDK proteins has been categorized into eight classes labeled CDKA-CDKG and cyclin-dependent kinases (CKL) (Tank and Thaker, 2011). Products of these genes are essential in, for example, G1/S and G2/M transitions (Hemerly et al., 1995), integration of developmental pathways (Boudolf et al., 2004), cell expansion in leaves and cell fates in floral organs (Inze and de Veylder, 2006), but the biological function of most CDKs remains largely unknown.

In *Arabidopsis* the CDKG class includes two putative kinases containing the conserved PLTSLRE motif (Tank and Thaker, 2011). The *CDKG;2* gene (ID At1g67580), encoding a product with a predicted molecular weight of 85.2 kDa, is located on the first chromosome (Swarbreck et al., 2008) and shows significant similarity to genes of animal and human PITSLRE kinases – $p34^{cdc2}$ family members (Kuta et al., 2008). In animals and humans, PITSLRE kinases play a role in RNA processing, regulation of transcription, apoptosis induction, oncogenesis, and dopamine/glutamate signaling in the nervous system (Knockaert et al., 2002; Trembley et al.,

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2004). A global expression analysis of cell cycle regulators revealed that *CDKG;2* in *Arabidopsis* is expressed predominantly in emerging seedlings and in suspension/callus cells (Menges et al., 2005), and therefore it has been suggested to play a role in cell differentiation processes (Tank and Thaker, 2011).

Arabidopsis thaliana (L.) Heynh is used as a model plant to study different processes including genetic control of plant development (Bowman et al., 2012; Enugutti et al., 2012; Petricka et al., 2012 and references therein), response to biotic and abiotic stresses and the genetics of local adaptation (Gaut, 2012). It also is a model in plant proteome and genome research (Joshi et al., 2012; Liu et al., 2012; Yandell and Ence, 2012 and references therein).

Work employing in vitro culture of *Arabidopsis thaliana* began in the 1970s, focusing on direct and indirect organogenesis from different explants in various media (Negrutiu et al., 1975; Masson and Paszkowski, 1992; Gaj 2001a and references therein). More recently, in vitro technique has been applied to study somatic embryogenesis in *Arabidopsis* ecotype Columbia and mutants (Gaj 2001a,b, 2004; Gaj et al., 2005, 2006; Kurczyńska et al., 2007; Fraś et al., 2008; Baster et al., 2009; Kraut et al., 2011; Nowak et al., 2012). Studies of the effect of in vitro culture conditions on *MEA* and *FIE/FIS* gene expression analyzed induction of egg cell and central cell divisions without fertilization in cultures of unfertilized ovules of *Arabidopsis* ecotypes (Columbia, Landsberg) (Rojek et al., 2005; Kapusta et al., 2007).

In vitro culture techniques enable laboratory study of the effect of mutation on cell division, tissue differentiation, organogenesis and somatic embryogenesis. Mutants with plant hormone response defects (Catterou et al., 2002; Gaj et al., 2006; Qiao et al., 2012), embryo-lethal mutants (Baus et al., 1986), and *lec* mutants controlling *Arabidopsis* zygotic embryogenesis (Gaj et al., 2005) have been studied in vitro to assess the biological function of genes involved in developmental process regulation.

In this study we characterized callus induction and subsequent plant regeneration in vitro in an *Arabidopsis* insertional T-DNA knockout line with inactivated *CDKG;2* in order to determine whether *CDKG;2* is required for organogenesis and/or cell proliferation in culture

MATERIALS AND METHODS

PLANT MATERIAL AND SELECTION OF THE KNOCKOUT LINE

Arabidopsis seeds of the parental line Columbia-0 ecotype (CS60000, referred to as Col-0) and N590262 (T_3 generation of T-DNA insertional line SALK_090262; Alonso et al., 2003) were obtained from the Nottingham *Arabidopsis* Stock Centre (UK). Plants were cultivated in soil under standard conditions: 22±2°C, 65% RH, and 12 h photoperiod under daylight fluorescent lamps (Sylvania, Luxline plus; fluence rate 80–120 $\mu mol \cdot m^{-2} s^{-1}$).

The homozygous line was isolated at generation T_4 based on PCR mapping (Young et al. 2001) using primers complementary to the flanking position of the coding region of full-length cDNA clone RAFL-07-13-L20 obtained from RIKEN (Tsukuba, Japan) (Seki et al., 1998; Seki et al., 2002): (cdkg2-for: 5' ATGGCGGCTGGGAGGAATATAA3'; cdkg2-rev: 5'T-CAGCCAAACAGACCGCCAGA3') and to flanking regions of the T-DNA insert (LBa1: 5'TGGTTCACG-TAGTGGGCCATCG3', Rba1: 5'CCAAACGTAAAAC-GGCTTGT3') in generation T_4. Homozygosity was tested in generation T_5. All PCRs were done with high-fidelity Marathon polymerase (AA Biotechnology, Gdynia, Poland). The position and orientation of the T-DNA insert were identified after sequencing of selected PCR products. DNA was isolated with a plant genomic DNA isolation kit (AA Biotechnology). Isolation of total plant RNA and RT-PCR was performed using commercial kits (Fermentas, LT).

EXPLANT ISOLATION FOR IN VITRO CULTURE

Seeds of both genotypes were sterilized by vortexing in 70% EtOH for 3–5 min and then in 50% solution of commercial bleach (Ace) with added Triton X-100 detergent for 3–5 min, followed by 3 rinses in sterile distilled water. Sterile seeds were placed in sterile Petri dishes filled with 0.15% solution of agar (MP Biomedicals), sealed with parafilm and refrigerated at 4°C for 3 days. Culture media and tools were autoclaved for ~30 min in a steam autoclave (121°C, pressure 1.05 bar).

Hypocotyls and cotyledons of 3-day-old seedlings of both genotypes were excised under a laminar flow hood and placed in medium-filled Petri dishes (10 explants per dish); 620 hypocotyls and 660 cotyledons were cultured in vitro. The experiment was repeated five times in 2009–2012.

CULTURE MEDIA AND CONDITIONS

The explants of both genotypes were cultured initially on MS medium (Murashige and Skoog, 1962) containing 3% sucrose (w/v), solidified with 0.8% agar (w/v) and supplemented with 2,4-dichlorophenoxyacetic acid (2 mg l^{-1} 2,4-D, Fluka), medium pH 5.7–5.8, for callus induction and proliferation. After 4 weeks, non-morphogenic callus was transferred to MS basal medium supplemented with thidiazuron (0.5 mg l^{-1} TDZ, Sigma-Aldrich). Regenerated shoots were rooted on MS + 0.5 mg l^{-1} IBA (Sigma) or on half-strength MS with reduced sucrose concentration (2% w/v). Then the cultures

were transferred to fresh medium at 2–3-week intervals. Cultures were maintained in a growth chamber at 25±3°C under a 16 h photoperiod (cool-white fluorescent lamps, 60–90 μmol·m^{-2}s^{-1}). Rooted plantlets were transplanted to plastic pots with an autoclaved 3:1 mixture of garden soil and perlite and kept in a plastic mini-greenhouse for 2 weeks. The hardened plants were maintained under room conditions until flowering and seed set.

HISTOLOGICAL ANALYSIS

Explants and callus fragments were fixed in 10% glutaraldehyde for 24 h, washed 4 times in phosphate buffer (PBS; pH=7.2) and then dehydrated in a graded ethanol series (10%, 30%, 50%, 70%, 96%; 15 min each) and kept overnight in absolute ethanol. Fixed tissue samples were embedded in Technovit 7100 (2-hydroxyethyl-methacrylate) (Heraeus Kulzer) followed by infiltration in a mixture of absolute ethanol and Technovit (3:1, 1:1, 1:3 v/v; 1 h each) and finally stored for 12 h in pure Technovit. The resin was polymerized with the addition of hardener. The material was sectioned 5 μm thick with a rotary microtome (Microm, Adamas Instrumenten), stained with toluidine blue and mounted in Entellan (Merck). LM sections were photographed with a Zeiss Axio Cam MRc digital camera with Zeiss Axio Vision 3.1 software.

SCANNING ELECTRON MICROSCOPY

Calluses of Col-0 and mutant explants were prefixed in 5% buffered glutaraldehyde (0.1 M phosphate buffer, pH 7.2) for 2 h at room temperature. After dehydration in a graded ethanol series, samples were CO_2 critical-point dried (EMITECH K850 system), sputter-coated with gold (SPI SUPPLIES ionsputtering system) and observed with a PHILIPS XL 30 scanning electron microscope.

RESULTS

We selected the *Arabidopsis* knockout *cdkg;2* line from generation T_4 based on PCR analysis using primers cdkg2-for and cdkg2-rev, complementary to flanking positions of the coding region of its full-length cDNA clone isolated by Seki et al. (2002). In Col-0 genomic DNA, PCR with cdkg2-for and cdkg2-rev primers gave a single 3.3 kbp product attributable to the *CDKG;2* coding sequence. This product was shown to be absent from the *cdkg;2* line when genomic DNA was used as template (Fig. 1a). Further analysis using combinations of these primers and primers complementary to flanking regions of T-DNA showed the presence of a T-DNA insertion within the *CDKG;2* coding sequence (Fig. 1b). The homozygosity of the isolated line was confirmed in generation T_5. The position of the T-DNA insertion localized in the first exon of the *CDK;2* sequence (Fig. 1c). The T-DNA insertion prevented accumulation of the specific transcript (data not shown). The knockout *cdkg;2* plants grown in soil under standard conditions produced viable seeds. The *cdkg;2* plants produced slightly larger rosettes and greener leaves than Col-0 (Fig. 1d,e).

Callus induction and proliferation was achieved on MS + 2 mg l^{-1} 2,4-D. The response of hypocotyl and cotyledon explants of Col-0 and *cdkg;2* was similar, but callus proliferation was earlier in mutant than in Col-0 explant culture: in mutant culture, 8.7% of the hypocotyl and 44% of the cotyledon explants started to produce callus after 3 days; proliferation of callus tissue on Col-0 explants was noted at day 7 of culture (Tab. 1).

The callus was compact and whitish or bicolor whitish green (Fig. 2). Neither roots nor shoots formed on auxin-supplemented medium. On some explants of the Col-0 ecotype and *cdkg;2*, hair-like structures appeared on the tissue surface (Fig. 2 a,e,k).

Shoots were formed after transfer of 21- and 28-day-old callus to MS medium supplemented with TDZ (0.5 mg l^{-1}). On Col-0 hypocotyl-derived callus the first shoots were observed after 7 days; in *cdkg;2* mutant culture the first shoots were not observed until day 31 of culture. The frequency of mutant explants producing shoots (6.8%) was much lower than for Col-0 (63.9%). Shoots did not form on Col-0 cotyledon-derived callus, whereas 12.8% of the mutant explants developed shoots after 31 days of culture (Tab. 2; Fig. 3). The number of shoots on hypocotyl-derived callus of Col-0 was very high: the whole explant surface was covered by developing shoots (Fig. 3a). The frequency of shoot formation was higher on *cdkg;2* explants at the end of culture (Tab. 2).

Histological analysis of Col-0 hypocotyl-derived callus after 28 days of culture on MS + TDZ showed meristematic centers forming shoot apices (Fig. 4a,b), sporadic embryo-like structures (Fig. 4b), and organs visible in transverse section (Fig. 4c). In contrast, hypocotyl-derived callus of the *cdkg;2* mutant was heterogenous, with parenchymatous cells, differentiated xylem elements, large vacuolized cells at the periphery of callus tissue (Fig. 4d,e), and easily identified groups of small meristematic cells scattered within the callus tissue (Fig. 4e). The surface of Col-0 and mutant hypocotyl- and cotyledon-derived calluses cultured 28 days on MS + TDZ was covered with a membranous structure resembling extracellular matrix (ECM) (Fig. 5).

Twelve regenerated shoots of Col-0 formed on hypocotyl-derived callus were rooted on MS + IBA or 1/2 MS + 2% sucrose and then acclimatized.

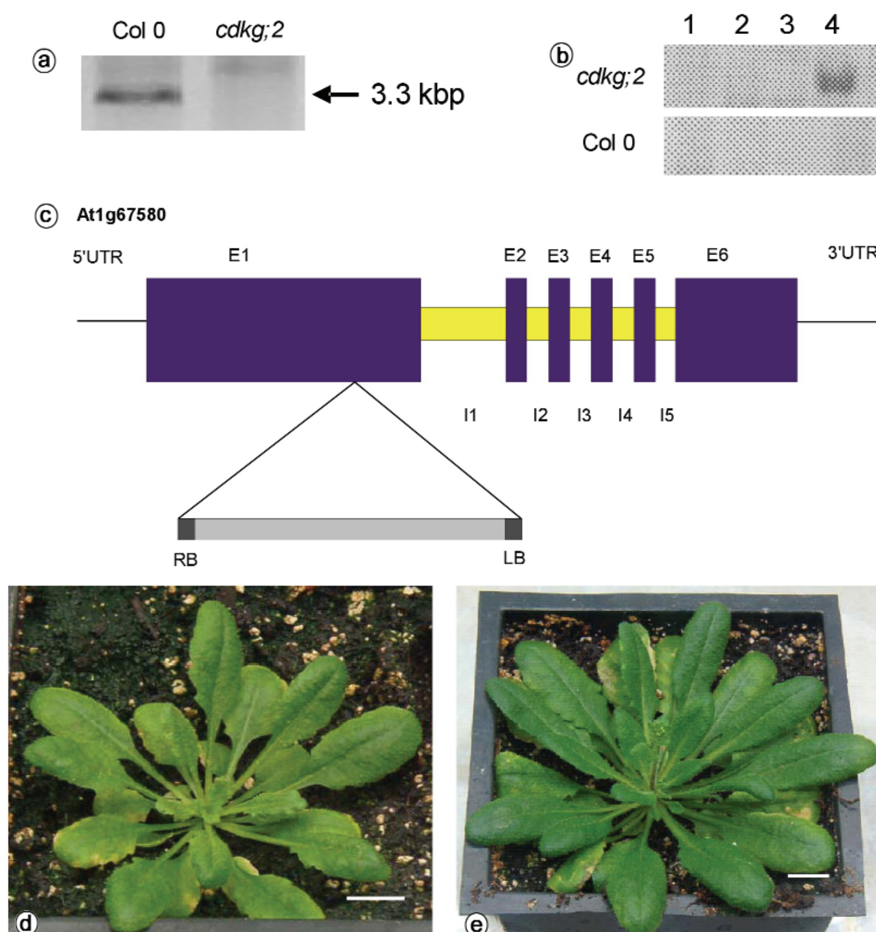

Fig. 1. Selection of the knockout *cdkg;2* line. (**a**) Presence and absence of 3.3 kbp PCR product in Col-0 and *cdkg;2* genotypes, respectively. (**b**) Identification of T-DNA insert in *cdkg;2* line. Lane 1: cdkg2-for/LBb1, lane 2: cdkg2-for/Rba1, lane 3: cdkg2-rev/Lba1, lane 4: cdkg2-rev/Rba1. (**c**) Map of *CDKG;2*. In the selected *cdkg;2* knockout line, T-DNA is inserted in the first exon. 21-day-old mature plants of Col-0 (**d**) and *cdkg;2* mutant (**e**) grown in soil under controlled conditions. Bar = 1 cm.

TABLE 1. Effect of 2,4-D (2 mg l^{-1}) on callus production of *Arabidopsis thaliana* Col-0 and *cdkg;2* mutant explants. Values [%]) are averaged from five replicates

Day of culture	Col-0		*cdkg;2* mutant	
	No. of explants used*	No. of explants producing callus	No. of explants used*	No. of explants producing callus
Hypocotyls				
3	320	0	300	26 [8.7]
7	275	275 [100]	265	265 [100]
14	230	230 [100]	220	220 [100]
21**	215	215 [100]	210	210 [100]
Cotyledons				
3	330	0	330	146 [44]
7	300	273 [91]	295	284 [96]
14	260	260 [100]	260	260[100]
21**	250	250 [100]	235	235 [100]

* The number of explants declined on successive days due to explant necrosis and sampling for histological analysis.
** Some explants were cultured 28 days.

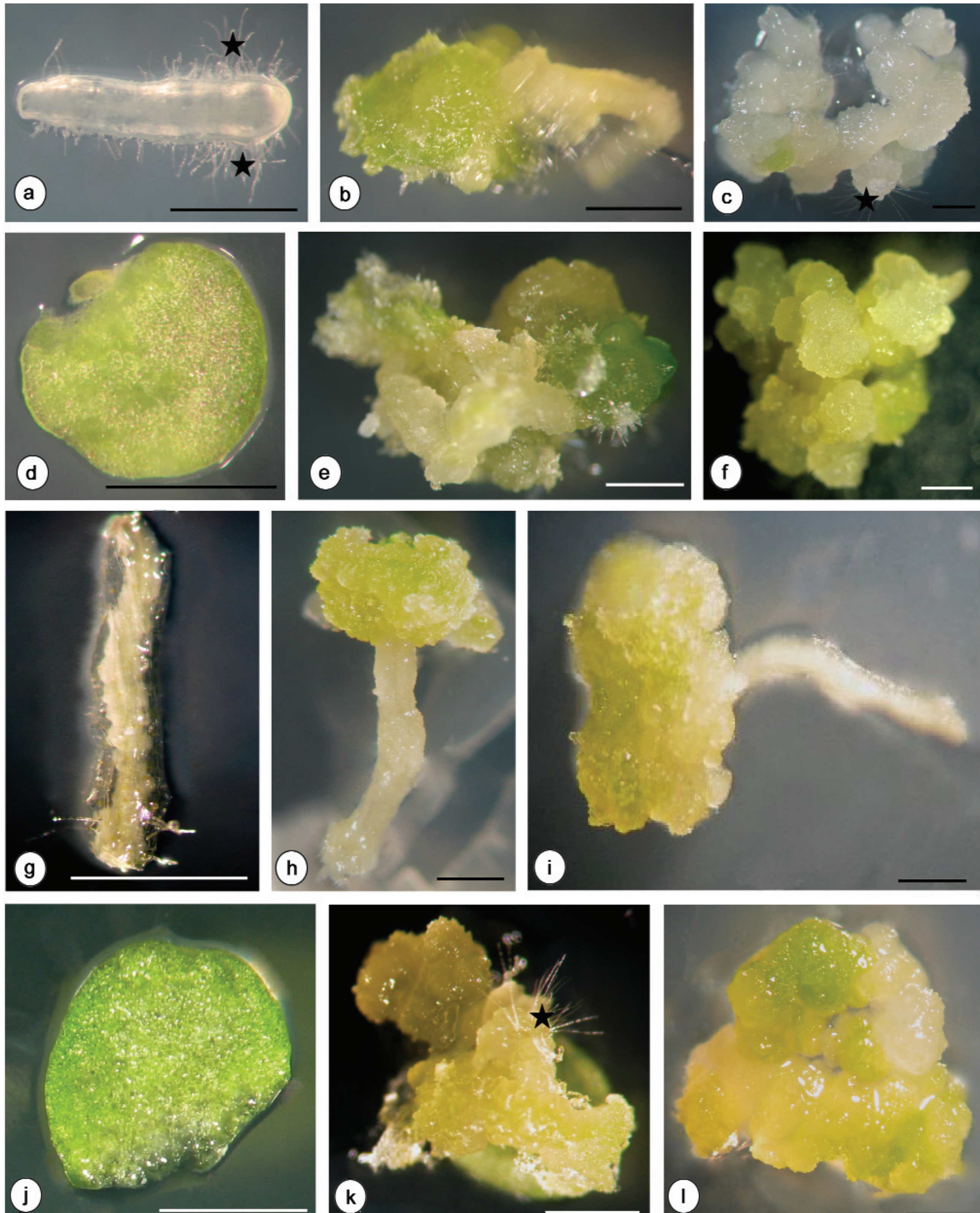

Fig. 2. *Arabidopsis thaliana* Col-0 (**a–f**) and *cdkg;2* mutant (**g–l**). Callus induction on MS + 2 mg l^{-1} 2,4-D on hypocotyls (**a–c; g–i**) and on cotyledons (**d–f; j–l**) after 3 days of culture (**a, d, ,j**), 14 days (**b, e, h, k**), 24 days (**i, l**), 28 days (**c, f**). Hair-like structures (**a,c,k**, stars), bicolor callus (**b, c, e, h, i, l**). Bar = 1 mm.

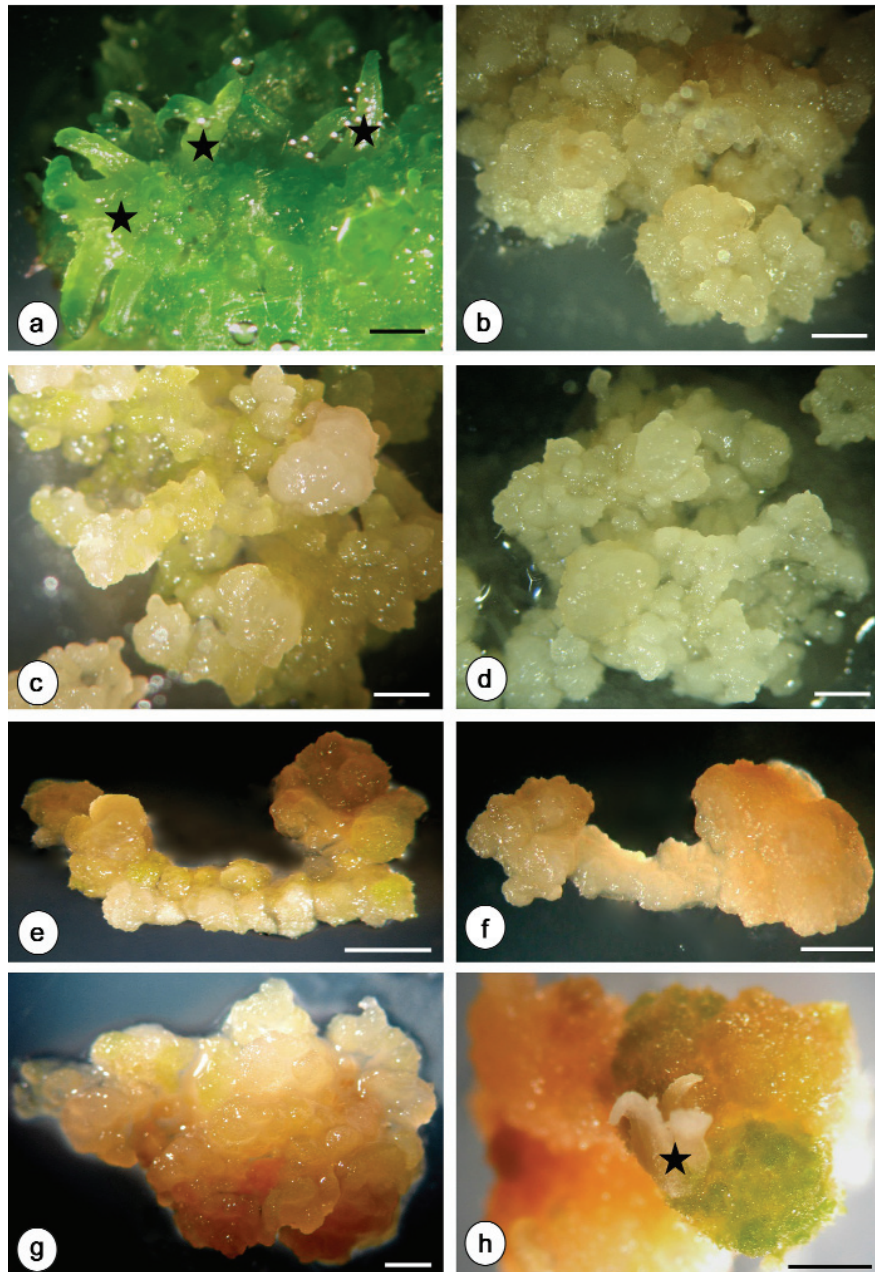

Fig. 3. *Arabidopsis thaliana* Col-0 (**a–d**) and *cdkg;2* mutant (**e–h**). Callus derived from hypocotyls (**a, b, e, f**) and cotyledons (**c, d, g, h**) on MS + 0.5 mg l^{-1} TDZ after 14 days of culture (**e, g**), 17 days (**a, c**), 31 days (**b, d, f, h**). Shoots are starred (**a, h**). Bar = 1 mm.

In *cdkg;2*, none of the 86 shoots from hypocotyl-derived callus and none of the 66 shoots from cotyledon-derived callus formed roots on rooting media. Acclimatized Col-0 plantlets developed flowers and produced seeds after self-fertilization (Fig. 6a-d). The rootless *cdkg;2* regenerants were dwarfed and their inflorescences had small budlike flowers with a reduced corolla and generative organs unable to yield seeds (Fig. 6e–h).

DISCUSSION

Cyclin-dependent kinases are known to be involved in the regulation of various developmental processes in plants. In this study we selected, for the first time, an *Arabidopsis* insertional mutant line with an inactivated *CDKG;2* gene encoding a putative cyclin-dependent protein kinase containing a conservative PLTLSRE motif. Mature plants of the homozygous

TABLE 2. Effect of TDZ (0.5 mg l^{-1}) on organogenesis in hypocotyl- and cotyledon-derived callus of *Arabidopsis thaliana* Col-0 and *cdkg;2* mutant. Values [%] are averaged from five replicates, except for one experiment which extended to 42 days

Day of culture	Col-0		*cdkg;2* mutant	
	No. of explants used*	No. of explants producing shoots	No. of explants used*	No. of explants producing shoots
Hypocotyls				
7	215	12 [5.6]	210	0
14	200	116 [58]	195	0
31	180	115 [63.9]	175	12 [6,8]
42	50	12 [24]	50	33 [66]
Cotyledons				
7	250	0	235	0
14	235	0	225	0
31	200	0	210	27 [12.8]
42	60	0	50	26 [52]

*The number of explants declined on successive days due to explant necrosis and sampling for histological analysis.

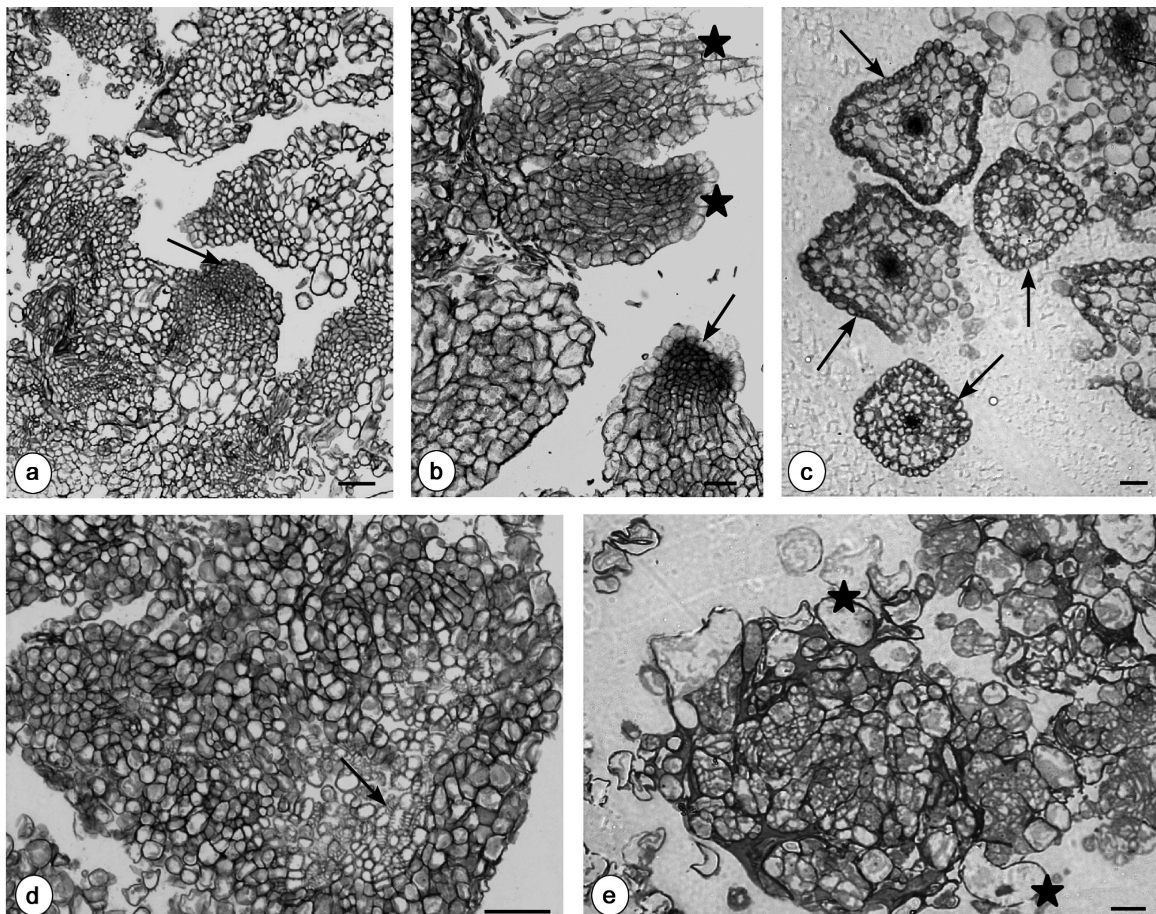

Fig. 4. *Arabidopsis thaliana* Col-0 (**a–c**) and *cdkg;2* mutant (**d, e**). Transverse sections of hypocotyls after 28 days of culture (**a–c**) and 31 days (**d, e**) on MS + 0.5 mg l^{-1} TDZ. (**a**) Meristematic region (arrow), (**b**) Embryo-like structures (stars) and meristematic region (arrow), (**c**) Transverse sections of regenerating organs (arrows), (**d**) Callus with parenchymatous cells and xylem elements (arrow), (**e**) Callus with groups of small meristematic cells scattered within tissue and large vacuolized cells at periphery of callus (stars). Bar = 100 μm.

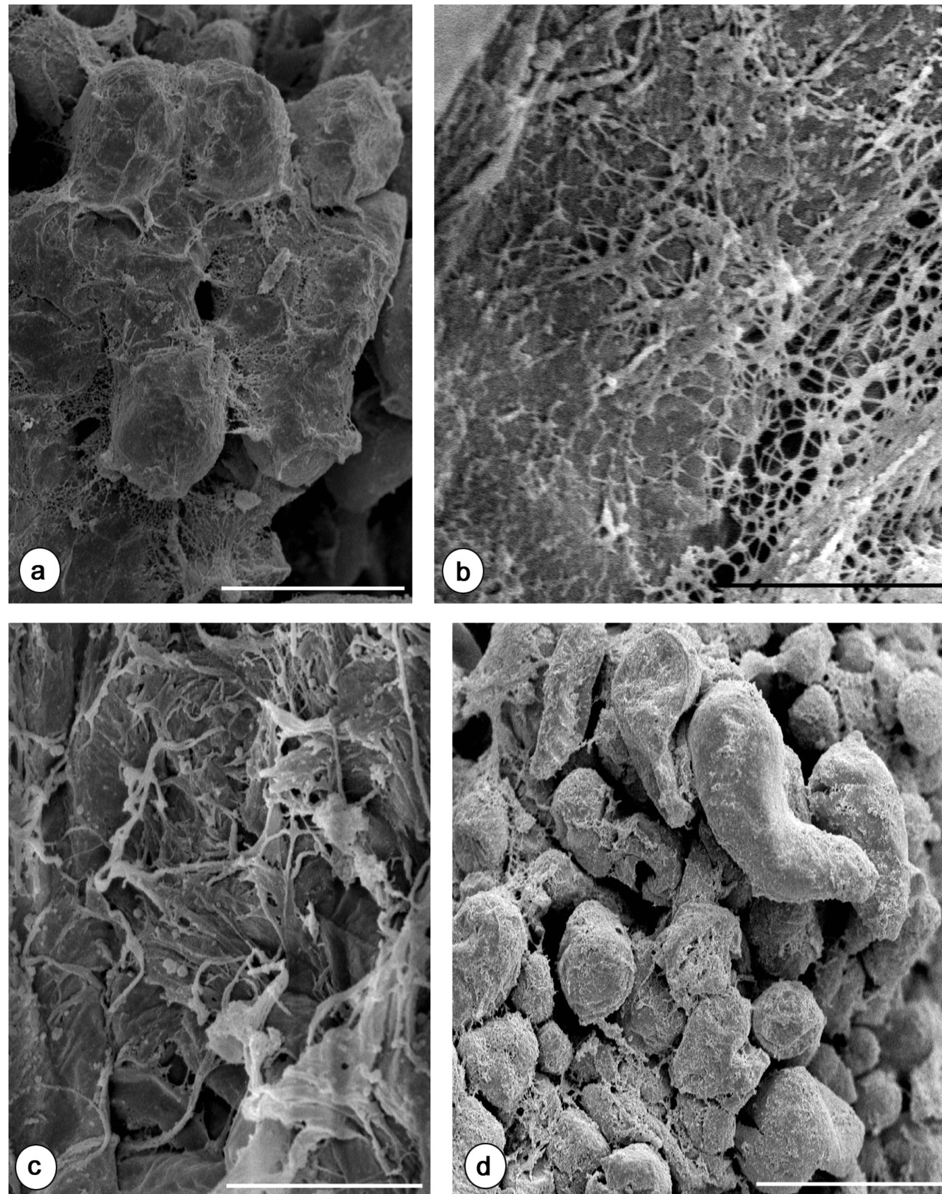

Fig. 5. *Arabidopsis thaliana* Col-0 (**a, b**) and *cdkg;2* mutant (**c, d**) callus induced on MS + 2 mg l^{-1} 2,4-D after 28 days of culture on MS + 0.5 mg l^{-1} TDZ. Membranous structure covering hypocotyl-derived (**a, c**) and cotyledon-derived (**b, d**) callus. Note the fibrillar structure of ECM densely (**b**) and partially (**d**) covering callus tissue. Bar = 30 μm (a), 6 μm (b), 8 μm (c), 60 μm (d).

cdkg;2 line showed no apparent phenotypical differences in morphology from their genetic background (Col-0), and completed the life cycle during growth in soil culture. This result suggests that the CDKG;2 function might be limited to specific tissues and/or processes in *Arabidopsis* development. Also, CDKG;2 may express functional overlapping with other CDK family members. Such overlapping between CDKA and CDKB kinases (Dissmeier et al., 2007) and between CDKF and CDKB (Takatsuka et al., 2009) has been demonstrated.

In *Arabidopsis thaliana* ecotype Columbia, the result of in vitro culture depends on the explant and medium type. Somatic embryogenesis was successfully induced from immature zygotic embryos cultured on Gamborg (B5) medium supplemented with 2,4-D (Gaj, 2001a,b; Kurczyńska et al., 2007; Fraś et al., 2008). Immature zygotic embryos are the ones mostly used as explants for direct and indirect formation of somatic embryos in various plant species, including *Trifolium nigrescens* (Konieczny et al., 2010), *Helianthus annuus* (Jach and Przywara,

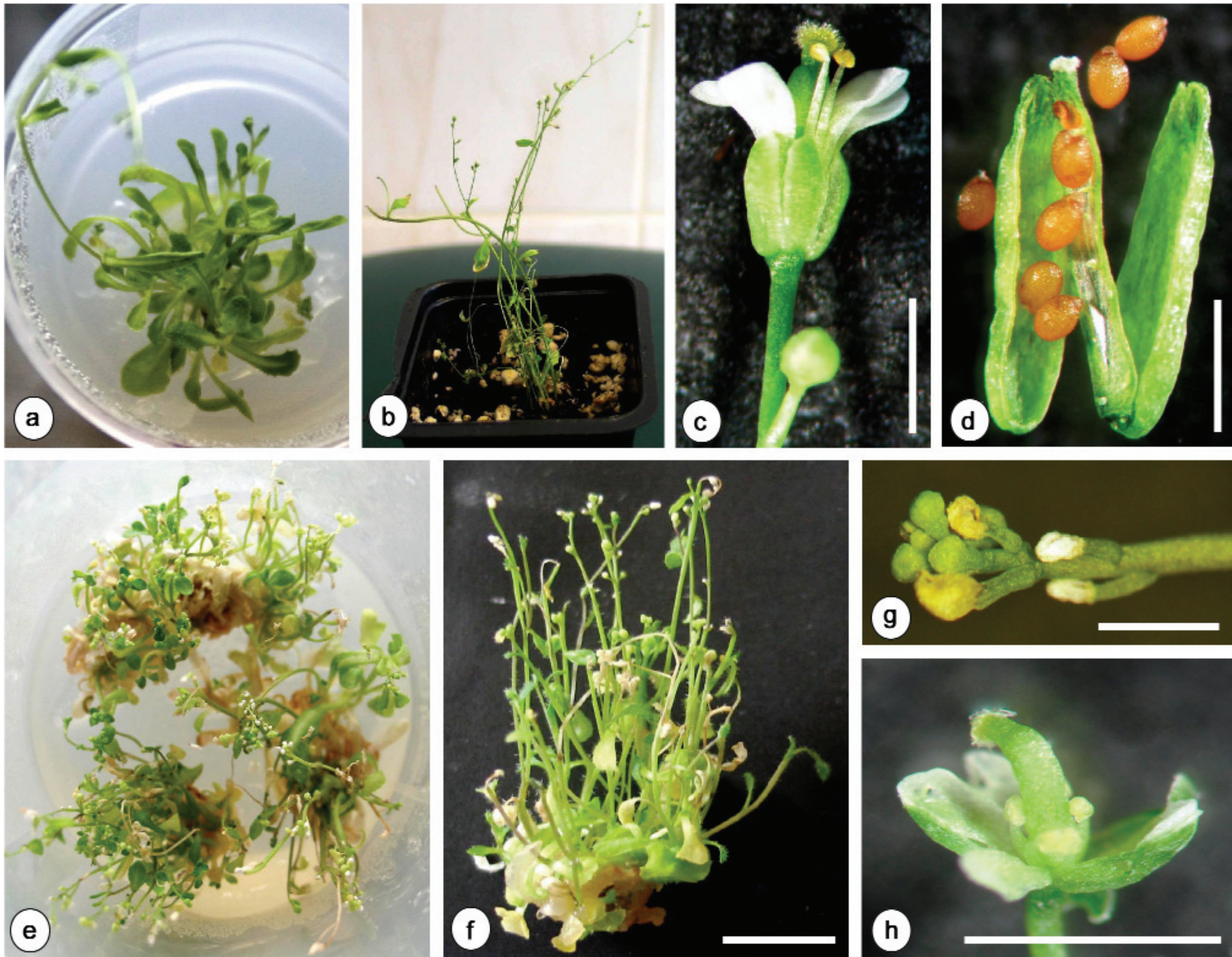

Fig. 6. *Arabidopsis thaliana* Col-0 (**a–d**) and *cdkg;2* mutant plants regenerated in vitro (**e–h**). (**a**) Rooting, (**b**) Acclimatization, (**c**) Flower, (**d**) Fruit with seeds, (**e, f**) Rootless regenerated plantlets, (**g**) Inflorescence, (**h**) Abnormal flower. Bar = 1 mm (c,d,g,h); 1 cm (f).

2000), *Sorbus pohuashanensis* (Yang et al., 2012) and *Zea mays* (González et al., 2012). In our work, using hypocotyls and cotyledons isolated from 3-day-old seedlings of Col-0 and a selected *cdkg;2* mutant line cultured on MS medium with 2,4-D, only callus induction and proliferation was achieved. Neither somatic embryos nor shoots/roots were formed on medium supplemented with the auxin only. This result stands in contrast to observations from other species cultured under the same experimental conditions. For example, in culture of *Brassica napus* cv. Kana cotyledons and hypocotyls on MS supplemented with 2,4-D, callus induction was accompanied by either caulogenesis or rhizogenesis, depending on the explant type (Ślesak et al., 2005).

There was no substantial difference in callus proliferation on MS medium with 2,4-D between Col-0 and *cdkg;2* explants. Callus proliferated on both Col-0 and *cdkg;2* explants, with higher intensity in *cdkg;2*, indicating that inactivation of that *CDKG;2* gene did not affect the cells' ability to divide.

Between Col-0 and the *cdkg;2* line there were significant differences in the course of organogenesis and plant regeneration from callus. Shoots started to develop on Col-0 hypocotyl-derived callus 7 days after it was transferred to medium with the cytokinin (TDZ), but not on cotyledons. In contrast, shoots were regenerated from *cdkg;2* callus after 31 days of culture in the same experimental conditions. In *Arabidopsis*, previously the *CDKG;2* product was found to accumulate predominantly in emerging seedlings and in suspension/callus cells (Menges et al., 2005). The delayed organogenesis of *cdkg;2* callus suggests that CDKG;2 kinase is involved in regulation of processes crucial for differentiation of plant cells.

The shoot regeneration frequency of hypocotyl-derived explants was lower in *cdkg;2* than in Col-0 after one month of culture. The regeneration rates of apical shoots from explants in vitro are highly variable and sensitive to both biotic and abiotic factors. In *Arabidopsis*, variability of long-term shoot regeneration was demonstrated to arise within the first hours post-excision from inadvertent, variable exposure of explants to light, modulated by hormones (Nameth et al., 2013). Shoot regeneration frequency has been shown to correlate positively with local cytokinin/auxin ratios in tissue cultures (Krikorian, 1995). Auxins play a key regulatory role in cotyledon development as a signalling determinant, so auxin synthesis and its concentration is critical to cotyledon organogenesis (Chandler, 2008). The absence of shoot regeneration on cotyledon explants (Col-0) or the reduction of its frequency (mutant) in this work could be an effect of a high concentration of endogenous auxin in cotyledon-derived callus. When cotyledon-derived callus already having a high level of endogenous auxin, cultured in callus-inducing medium with exogenous auxin (2,4-D), was then transferred to medium supplemented with a cytokinin (TDZ), it may have disturbed the auxin:cytokinin ratio required for shoot development. On the other hand, gradients of endogenous auxin concentrations formed along hypocotyls (Firml, 2003) could promote shoot regeneration from hypocotyl-derived callus via modification of the total auxin:cytokinin ratio in explants. The observed delay in adventitious shoot formation (after 31 days of culture) in the *cdkg;2* mutant when cotyledon-derived callus was transferred to the medium with cytokinin could be an effect of unknown developmental mechanisms regulated by CDKG;2 or could be the result of the cumulative effect of cytokinins from prolonged treatment on this medium.

The membranous structure covering Col-0 and mutant hypocotyl- and cotyledon-derived callus was similar to ECM reported in plant tissue culture of different species (Popielarska-Konieczna et al., 2010; Lai et al., 2011). A surface network of extracellular matrix is considered an early marker of morphogenetic processes, especially somatic embryogenesis induced in culture (Šamaj et al., 2006). In our experimental conditions, both Col-0 and *cdkg;2* callus cells were covered with ECM, irrespective of their morphogenetic ability.

In the *cdkg;2* mutant line, adventitious root formation on regenerated shoots was not observed on medium supplemented with an auxin (IBA), nor on MS without plant growth regulators. The normal development of *cdkg;2* seedlings after seed germination on different media (agar, MS, soil) clearly indicates that primary root development and lateral root formation is not affected by inactivation of the *CDKG;2* gene (Kuta et al., 2008). As auxins influence root morphology, increasing lateral root production, inhibiting root elongation and inducing adventitious roots (Woodward and Bartel, 2005), inactivation of *CDKG;2* seems not to disturb endogenous auxin production and transport. In plant tissue culture, auxins added to the media promote rhizogenesis in undifferentiated callus (Skoog and Miller, 1957). In our work, adventitious roots were not formed on MS supplemented with 2,4-D in callus derived from explants of *cdkg;2* seedlings, nor in plants regenerated from proliferating *cdkg;2* callus. This suggests that CDKG;2 activity is required for differentiation of root apical meristems in tissue culture.

The organogenesis we observed in the *cdkg;2* line in tissue culture resembles that process in explants of some embryo-lethal mutants. In particular, callus derived from arrested embryos of the lethal mutant 112A-2A failed to form roots on root-inducing media, and the embryos of this mutant developed into abnormal rootless plants when cultured in vitro. This phenotype has been associated with impaired development of the root apical meristem during organogenesis in vitro (Baus et al., 1986). Thus, the abnormalities observed during *cdkg;2* organogenesis suggest a role of CDKG;2 as a regulator of adventitious root meristem differentiation.

Here we showed that knocking out the gene encoding CDKG;2 kinase significantly affects morphogenic responses in culture. Organogenesis of the *cdkg;2* mutant on TDZ-supplemented media was delayed as compared to shoot formation in the Columbia ecotype. Roots did not form on rooting media. The development of regenerated shoots was disturbed, probably due to the absence of root differentiation. The rootless plantlets produced inflorescences but did not set seeds because the generative organs were not properly developed. Our results indicate that the function of CDKG;2 is not limited to cell cycle control but plays multiple roles both in early plant development (presumably in formation of the root meristem) and in triggering organ formation.

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