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Tracing meristem cell lineages during plant development and regeneration by the all-in-one CRE/LOX system

Shengnan Wang[1†], Yu Li[2,3†], Zijuan Li[2†], Chao Li[1*], Lin Xu[2*] and Ning Zhai[2*]

Abstract

The root apical meristem (RAM) and the shoot apical meristem (SAM), which contain stem cells and their lineages, are the centers for underground and aboveground organ formation in vascular plants. Accurately tracing stem cell lineage is important to understand how the RAM and SAM are formed and how they give rise to new organs. Here, we introduce the all-in-one *CRE/LOX* system, which is assembled in a single plasmid and can be used to efficiently trace cell lineage during *de novo* formation of meristems and cell differentiation within meristems in *Arabidopsis thaliana*. Using the *CRE/LOX-GUS* system driven by the *WUSCHEL-RELATED HOMEOBOX5* (*WOX5*) promoter (i.e., $WOX5_{pro}$*-CRE/LOX-GUS*), we show that cell division of the quiescent center (QC) within the stem cell niche of the RAM might replenish initial/stem cells in plants grown on mannitol-containing medium or in soil. The results obtained using $WOX5_{pro}$*-CRE/LOX-GUS* also show that a group of shoot progenitor cells acts together to initiate the SAM during *de novo* shoot regeneration from callus in tissue culture. We also demonstrate the use of the *CRE/LOX-RUBY* system for real-time in vivo tracing of cell lineages in live organs. Overall, this paper not only introduces the all-in-one *CRE/LOX* tool, but also reveals the cell lineages during meristem development and regeneration.

Keywords Plant regeneration, Cell lineage, Meristem, CRE/LOX, RUBY, *WOX5*, *WOX11*, *Arabidopsis thaliana*

Introduction

Plant meristems, which contain a group of undetermined and actively dividing cells, are the center of organ formation [1]. In vascular plants, underground and aboveground organs are initiated from the root apical meristem (RAM) and the shoot apical meristem (SAM), respectively. How the meristem is formed and how the meristem gives rise to new organs are key questions in plant development.

During post embryonic development of *Arabidopsis thaliana*, all the root tissues are differentiated from the RAM. The RAM is composed of the stem cell niche and transit-amplifying cells. The stem cell niche contains the quiescent center (QC) and the initial cells (i.e., stem cells) that surround the QC. Division of the initial/stem cells gives rise to transit-amplifying cells,

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and the daughter cells derived from transit-amplifying cell division differentiate to produce root tissues [1].

Arabidopsis can create root branches by *de novo* formation of the RAM [2, 3]. In acropetal lateral root (hereafter referred to as lateral root) formation, several xylem-pole pericycle cells function together as the lateral root founder cells that divide to initiate the lateral root primordium. The continuous division of the lateral root primordium forms the lateral root meristem [4]. In adventitious root formation from detached leaves (i.e., *de novo* root regeneration), a vascular adult stem cell near the wound serves as the adventitious root founder cell. This founder cell divides and initiates the adventitious root primordium, which gives rise to the adventitious root meristem *via* continuous cell division [5]. The *WUSCHEL* (*WUS*)*-RELATED HOMEOBOX* (*WOX*) family transcription factor gene *WOX11* is expressed in the adventitious root founder cell but not in lateral root founder cells, and its expression level decreases during the division of the adventitious root founder cell to form the adventitious root primordium [6–8]. The *WOX11* expression in the adventitious root founder cell is directly activated by the auxin signaling pathway through the auxin response elements (AuxREs) on the *WOX11* promoter [6]. In root meristems, *WOX11* is expressed in protoxylem cells [6, 7]. *WOX5* expression is activated during the transition from both lateral and adventitious root founder cells to the root primordium, and then its expression is enriched in the QC in root meristems [9–12]. WOX11 and the AP2-like transcription factors PLETHORA3/5/7 can directly bind to the *WOX5* promoter to active the *WOX5* expression during the fate transition from adventitious root founder cell to adventitious root primordium [11, 13].

In two-step tissue culture of *Arabidopsis* hypocotyls, the hypocotyl explants are first cultured on callus-inducing medium (CIM) with a high auxin level. This induces the explants to form pluripotent callus in preparation for organ regeneration. Then, the callus is moved to shoot-inducing medium (SIM) with a high cytokinin level to induce shoot regeneration [14, 15]. On CIM, a vascular adult stem cell within the hypocotyl explant serves as the callus founder cell, which expresses *WOX11* activated by auxin [6, 16, 17]. Then, the callus founder cell divides to form the callus primordium that expresses *WOX5* [6, 16–18]. Continuous cell division of the callus primordium gives rise to callus with three cell layers (i, e. the outer, middle, and inner cell layers), and *WOX5* is enriched in the middle cell layer [16, 17]. After the callus is moved to SIM, shoot progenitor cells form in the middle cell layer by expressing *WUS* [16, 19–24]. Then, shoot progenitor cells can enter into two fate directions: the productive shoot progenitor cells continuously divide, express SAM-related genes, and finally form the SAM, while the pseudo shoot progenitor cells lose the ability to form a SAM [24]. Although the SAM can be traced back to a single cell in the explant before culture on CIM [25], it is still unclear whether a single shoot progenitor cell or a group of shoot progenitor cells is responsible for SAM formation on SIM.

The developmental and regenerative processes described above provide efficient platforms for studying meristem cell lineages. The CRE/LOX system has been widely used to trace cell lineages in animals [26–28] and plants [16, 28–32]. In our previous studies, we used a CRE/LOX system comprising two constructs, i.e., *CRE* in one construct and *LOX* in another construct. The constructs were separately transformed into *Arabidopsis*, and the cell lineage was traced by crossing the two transformed *Arabidopsis* lines harboring each construct [29]. In this study, we report the all-in-one CRE/LOX system that combines *CRE* and *LOX* in one construct and utilizes the RUBY reporter [33] for real-time and in vivo cell lineage tracing in live organs.

Materials and methods

All-in-one CRE/LOX system construction

The all-in-one CRE/LOX system was constructed based on the two separate plasmids reported previously [29]. Firstly, the 35S promoter in *pCAMBIA1300-LOX2272-eGFP-NOS*$_{ter}$*-LOX2272-GUS-NOS*$_{ter}$ was replaced with the *Arabidopsis UBIQUITIN 10* (*UBQ10*) promoter (*UBQ10*$_{pro}$) [34] to create the *UBQ10*$_{pro}$*-LOX2272-eGFP-NOS*$_{ter}$*-LOX2272-GUS-NOS*$_{ter}$ fragment. Secondly, this fragment was inserted after *pBI101-intCRE-GR-HSP*$_{ter}$ to create *pBI101-CRE/LOX-GUS*. The *pBI101-intCRE-GR-HSP*$_{ter}$ plasmid contained a *intCRE-GLUCOCORTICOID RECEPTOR* (*GR*) sequence by inserting the first intron of castor bean *CATALASE1* (*CAT1*) intron [35] into *CRE* (*intCRE*) as well as the *HEAT SHOCK PROTEIN 18.2* (*HSP18.2*) terminator (*HSP*$_{ter}$) [36]. The *β-GLUCURONIDASE* (*GUS*) gene in *pBI101-CRE/LOX-GUS* was replaced with *RUBY* [33] to create *pBI101-CRE/LOX-RUBY*. The 5-kb *WOX5* promoter was cloned into *pBI101-CRE/LOX-GUS* to create *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* and the 5-kb *WOX11* promoter was cloned into *pBI101-CRE/LOX-RUBY* to create *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY*.

The constructs were introduced into the wild-type *Arabidopsis* Columbia-0 (Col-0) via *Agrobacterium tumefaciens*-mediated transformation. Two from a total of twelve transgenic *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* lines and five from a total of sixteen transgenic *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY* lines showed

normal DEX-induced *GUS* and *RUBY* expression, respectively. The homozygous plants from those normal DEX-induced *GUS* or *RUBY* expression lines all showed reporter activities. Therefore, these lines were used in this study.

The forward and reverse primers used for cloning are listed below: 5′-GAATTCTCTAGAGAGTCAGTAATAAACGG-3′ and 5′-ACTAGTAAGCTTCTGTTAATCAGAAAAAC-3′ for the *UBQ10* promoter; 5′-ACTGATTTCGACCAGGTAAATTTCTAGTTTTTCTC-3′ and 5′-CATGAGTGAACGAACCTGTAACTATCATCATCATC-3′ for the *CAT1* intron; 5′-TCTAGAATATGAAGATGAAGATGAAA-3′ and 5′-ACTAGTCTTATCTTTAATCATATTCC-3′ for the *HSP18.2* terminator; 5′-ACAATTACCAACAACAACAAAC-3′ and 5′-CGACTCACTATAGGGTCTAGTCCCGATCTAGTAACATAGATGAC-3′ for *RUBY*; 5′-ACGCGTCGACTCAGAGACCAAATTATTTTGG-3′ and 5′-GGTACCGTTCAGATGTAAAGTCCTCAACTG-3′ for the *WOX5* promoter; and 5′-TTCGGAGAGCTCGGAGGTACCTGAGCTCATCTAACTGTTACG-3′ and 5′-CATAGATCTGCTAGCGTCGACTGCTTTGAAGAATATTGATAT-3′ for the *WOX11* promoter.

Plant materials, plant growth, regeneration conditions, and GUS staining assay

The $WOX5_{pro}$:*GUS* line was described previously [37].

For adventitious root regeneration from detached leaves, *Arabidopsis* seeds were germinated and grown on ½ Murashige & Skoog basal (MS) medium with 1% w/v sucrose at 22 °C under a 16-hour light/8-hour dark photoperiod for 12 days. The first pair of rosette leaves from 12-day-old seedlings was cut at the petiole, and the detached leaves were cultured on B5 medium without sucrose at 22 °C under 24-hour light conditions.

For shoot regeneration in tissue culture, *Arabidopsis* seeds were germinated and vertically grown on ½ MS medium (1.2% w/v agar) in the light for 1 day and then in the dark for 8 days. Elongated hypocotyls (about 1 cm) were cut and incubated on CIM [MS medium, 2% w/v sucrose, pH 5.8, 0.8% w/v agar, 11 μM 2,4-dichlorophenoxyacetic acid (2,4-D), 0.2 μM kinetin] under 24-hour light conditions for 5 days and 30 min to induce callus formation. Callus was transferred to SIM [MS medium, 2% w/v sucrose, pH 5.7, 0.8% w/v agar, 2 μM 6-(dimethylallylamino) purine (2iP), 0.9 μM indole acetic acid (IAA)] and cultured under 24-hour light conditions for 7 days.

For analyses of lateral roots and RAMs in plants growing on medium, *Arabidopsis* seeds were germinated and grown vertically on ½ MS medium containing 1% w/v sucrose at 22 °C under a 16-hour light/8-hour dark photoperiod.

For analyses of RAMs in plants grown in soil, *Arabidopsis* seeds were germinated and grown in soil at 22 °C under a 16-hour light/8-hour dark photoperiod.

Details of the GUS staining method have been reported previously [37].

Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative under the following accession numbers: *WOX5* (AT3G11260), *WOX11* (AT3G03660), *HSP18.2* (AT5G59720), and *UBQ10* (AT4G05320).

Results

All-in-one *pBI101-CRE/LOX-GUS* system

In our previous study, we used a CRE/LOX system based on two constructs [29]. Here, we combined all those elements into one construct based on the *pBI101* plasmid (Fig. 1A and B). The all-in-one construct *pBI101-CRE/LOX-GUS* consisted of two parts. First, the *intCRE* gene (*CRE* gene with an intron insertion to avoid accumulation of its encoded protein in bacteria) was fused with a dexamethasone (DEX)-induced nuclear localization domain *GR* sequence [38]. This *intCRE-GR* fused gene was terminated with the HSP_{ter} [36]. Tissue-specific promoters can be inserted into the multiple cloning site to drive the expression of *intCRE-GR*. Second, the fused fragment *LOX2272-eGFP-*NOS_{ter}*-LOX2272* was inserted between the $UBQ10_{pro}$ [34] and the *GUS* reporter gene to block the expression of *GUS* driven by $UBQ10_{pro}$.

To use this all-in-one *pBI101-CRE/LOX-GUS* system, a tissue-specific promoter must be cloned into the multiple cloning site. After the construct is transformed into plants, the *intCRE-GR* gene is transcribed under the control of the tissue-specific promoter. To trace cell lineage, DEX is used to allow the CRE-GR protein into the nuclei, then CRE-GR implements the recombination of the two *LOX2272* sequences to form a single *LOX2272* sequence, resulting in the activation of *GUS* expression. In this way, cells with the GUS signal are confirmed as descendants of the cell(s) in which the initial *LOX2272* recombination event occurred (Fig. 1A).

To test this system, we cloned the *WOX5* promoter into *pBI101-CRE/LOX-GUS* to form *pBI101-*$WOX5_{pro}$*-CRE/LOX-GUS* and transformed this construct into the wild-type *Arabidopsis* Col-0 background. The *pBI101-*$WOX5_{pro}$*-CRE/LOX-GUS* transgenic line was grown vertically on ½ MS medium for 7 days without DEX treatment, and then moved to DEX-containing medium and grown for another 3 days. The results show that some lateral roots were clearly labeled with the GUS signal (Fig. 2A). When developing

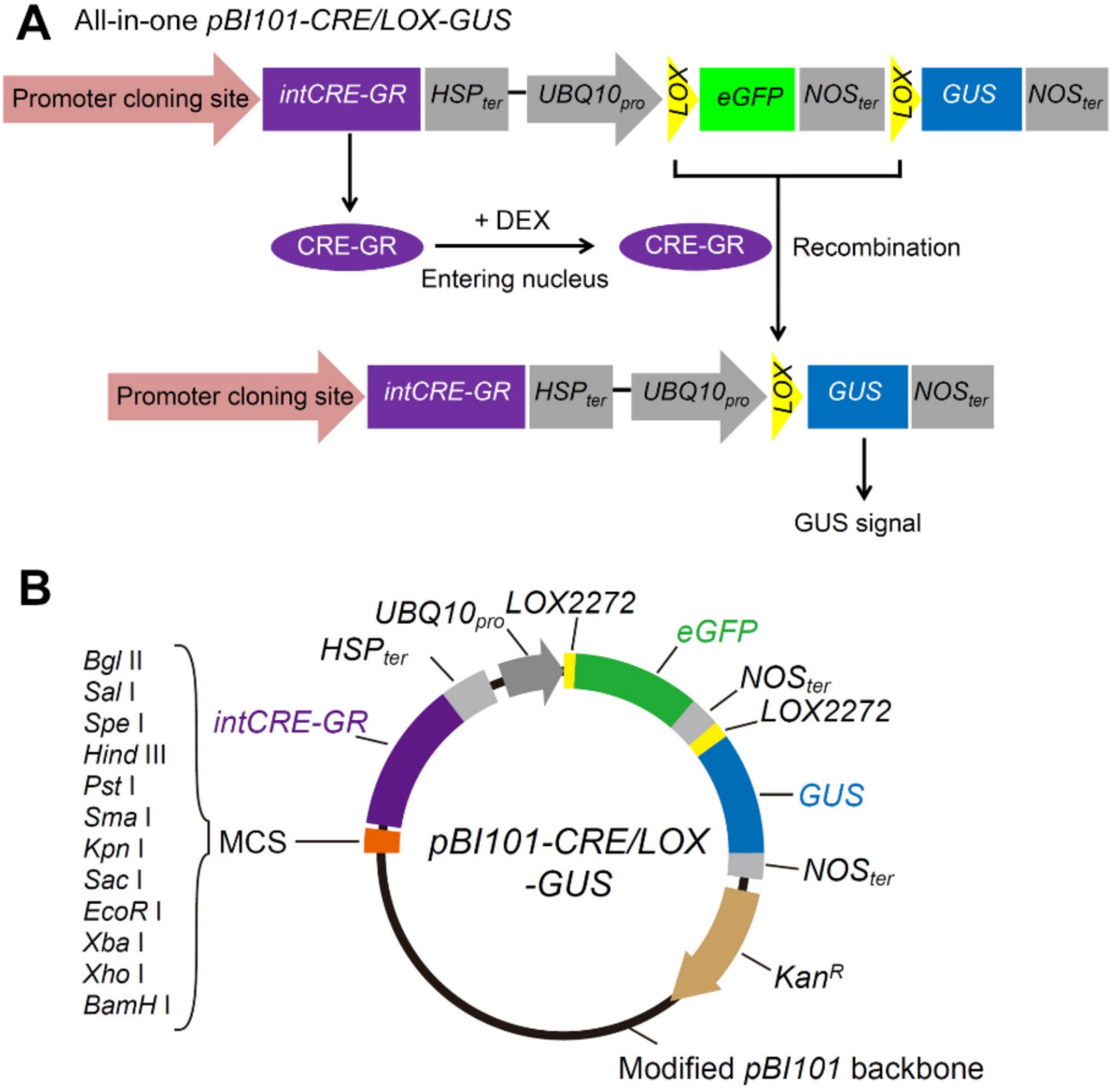

Fig. 1 The *pBI101-CRE/LOX-GUS* system. **A**. Schematic of the all-in-one *CRE/LOX-GUS* system to trace cell lineage. **B**. The plasmid vector *pBI101-CRE/LOX-GUS*. MCS, multiple cloning site

lateral roots were observed under a differential interference contrast (DIC) microscope, GUS signals were observed at all stages of lateral root primordia and the lateral root meristem (Fig. 2B–E). This result confirms that *WOX5* expression is activated in the lateral root primordium, and all the cells of the lateral root are descendants of the *WOX5*-expressing lateral root primordium [12, 29].

We also tested the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* system during adventitious root regeneration from detached *Arabidopsis* leaves (Fig. 2F). The detached leaves of the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* transgenic line were cultured on B5 medium with DEX for 7 days, and all the regenerated adventitious roots were labeled with GUS signals (Fig. 2G). In the control (no DEX), there were no GUS signals in the adventitious roots (Fig. 2G). These observations confirm the findings of previous studies, i.e., that *WOX5* is activated in the adventitious root primordium, and that all the cells of the adventitious root are descendants of the *WOX5*-expressing adventitious root primordium [11, 29].

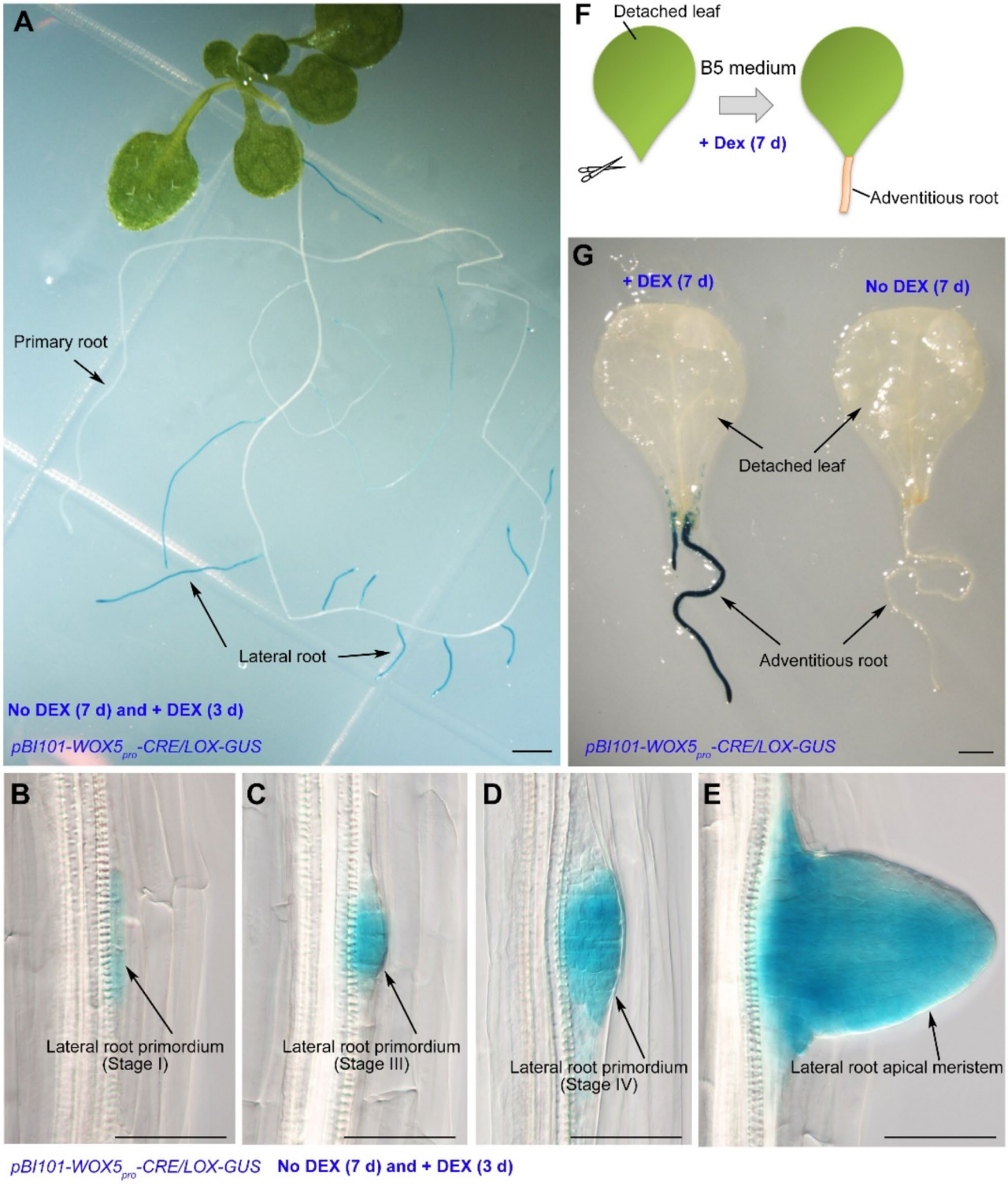

Fig. 2 Tracing lateral and adventitious root cell lineages using the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* system. **A–E**. GUS staining of the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* transgenic line, showing positive GUS signals in lateral roots (A), lateral root primordia (B–D), and lateral root meristem (E). Seedlings were germinated and grown vertically on ½ MS DEX-free medium (no DEX) for 7 days (d), then cultured on ½ MS medium containing 10 μM DEX (+ DEX) for 3 days. **F**. Diagram of adventitious root formation from detached *Arabidopsis* leaves in (G). **G**. GUS staining of the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* transgenic line, showing positive GUS signals in adventitious roots growing from detached leaves. Seedlings were cultured for 12 days on DEX-free ½ MS medium, and then two leaves (first leaf pair) were detached from the same plant. One was cultured on B5 medium with 10 μM DEX (+ DEX) for 7 days, and the other was cultured on B5 medium without DEX (no DEX) for 7 days as the control. Two independent lines were analyzed and showed the same results. Scale bars, 1 mm in (A, G) and 50 μm in (B–E)

Overall, the all-in-one *pBI101-CRE/LOX-GUS* is a reliable system showing the same results compared with the previous reported two-construct *CRE/LOX-GUS* system [29] in lateral and adventitious rooting.

Cell lineages in the RAM cultured on medium or in soil

Next, we analyzed the cell lineage in the RAM. The *pBI101-WOX5$_{pro}$-CRE/LOX-GUS* transgenic plants were cultured vertically on DEX-free ½ MS medium without mannitol for 3 days and then moved to DEX-containing ½ MS medium without (mock) or with mannitol to impose osmotic stress for another 2 days (Fig. 3A). The primary roots in the plants treated with mannitol were shorter than those in the plants with the mock treatment (Fig. 3B–D).

Under mock conditions, GUS signals were observed in the QC, indicating that the *WOX5*-marked QC cells did not divide to replenish initial/stem cells (Fig. 3E). Interestingly, in the mannitol treatment, the GUS signals in the *pBI101-WOX5$_{pro}$-CRE/LOX-GUS* line

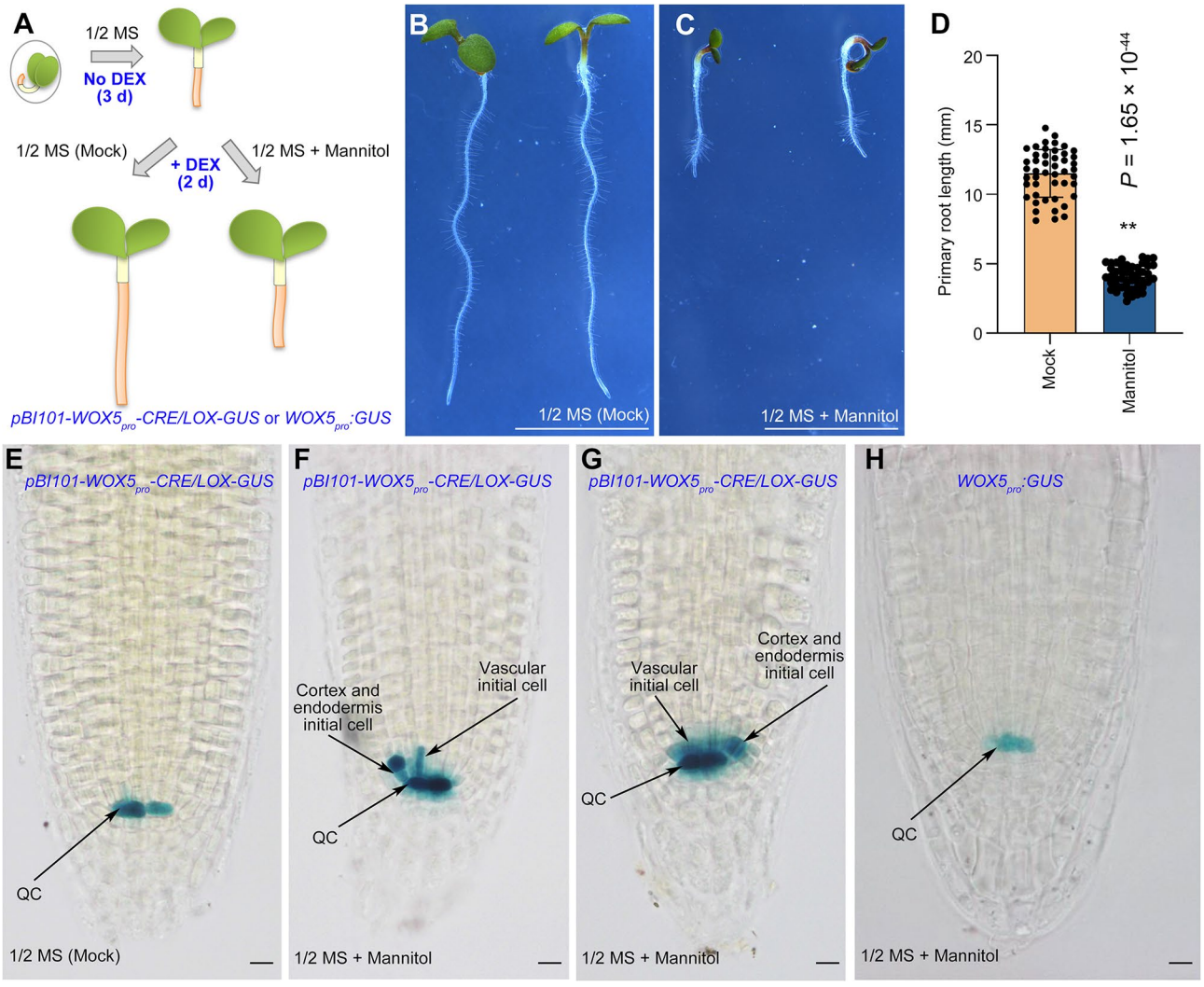

Fig. 3 Cell lineage in the stem cell niche of RAM under osmotic stress. **A**. Diagram of mannitol treatment to impose stress. *pBI101-WOX5$_{pro}$-CRE/LOX-GUS* transgenic seedlings were grown vertically on DEX-free ½ MS medium (no DEX) for 3 days (d). Then, seedlings were transferred to ½ MS medium containing 10 μM DEX (+DEX) containing 400 mM mannitol or without mannitol (0 mM mannitol as the mock) and grown for a further 2 days (B–H). **B–D**. Phenotype (B, C) and statistical (D) analyses of the *pBI101-WOX5$_{pro}$-CRE/LOX-GUS* transgenic seedlings grown in mock or osmotic stress conditions. Data are mean values ± SD ($n = 47$ in mock and $n = 46$ in mannitol treatment). ** $P < 0.01$ in two-tailed Student's t tests, and individual values (black dots) and mean values (bars) are shown (D). **E–G**. GUS staining in the *pBI101-WOX5$_{pro}$-CRE/LOX-GUS* transgenic line cultured with 0 mM (E, mock) and 400 mM (F, G) mannitol. Note that GUS signals were restricted to QC cells in 0 mM mannitol treatment (mock) (E) but were present in QC cells, vascular initial cells, and cortex and endodermis initial cells in mannitol treatments (F, G). A total of 9 roots were observed in mock conditions, and all of them showed GUS signals in the QC cells; and a total of 19 roots were observed under 400 mM mannitol treatment, and 9 of them showed GUS signals in initial cells. **H**. GUS staining in the *WOX5$_{pro}$:GUS* transgenic line cultured with 400 mM mannitol. A total of 20 roots were observed, and all of them showed GUS signals in the QC cells. Two independent lines were analyzed and showed the same results. Scale bars, 5 mm in (B, C) and 10 μm in (E–H)

were present in the vascular initial cells and cortex and endodermis initial cells in addition to the QC cells (Fig. 3F and G). However, in the mannitol treatment, the GUS signals in the $WOX5_{pro}$:*GUS* line were still observed in the QC (Fig. 3H). Therefore, these data indicated that the *WOX5*-marked QC cells might divide to replenish the initial/stem cells.

We also cultured the *pBI101-*$WOX5_{pro}$*-CRE/LOX-GUS* and $WOX5_{pro}$:*GUS* lines in soil without DEX for 3 days, and then with DEX for another 4 days (Fig. 4A and B). The GUS signals in the $WOX5_{pro}$:*GUS* line were enriched in the QC cells (Fig. 4E); but those in the *pBI101-*$WOX5_{pro}$*-CRE/LOX-GUS* line were present in cortex and endodermis initial cells, the columella stem cells, and the columella cells in addition to the QC cells (Fig. 4C and D). Thus, the *WOX5*-marked QC cells divided to replenish initial/stem cells when the plants were grown in soil.

Cell lineages in SAM regeneration during tissue culture

In the two-step tissue culture of *Arabidopsis* hypocotyls, *WOX5* expression is enriched in the middle cell layer of callus, where shoot progenitor cells may arise [16–18]. To analyze the cell lineage during SAM formation from callus in tissue culture, we established two different culture conditions. In the first condition,

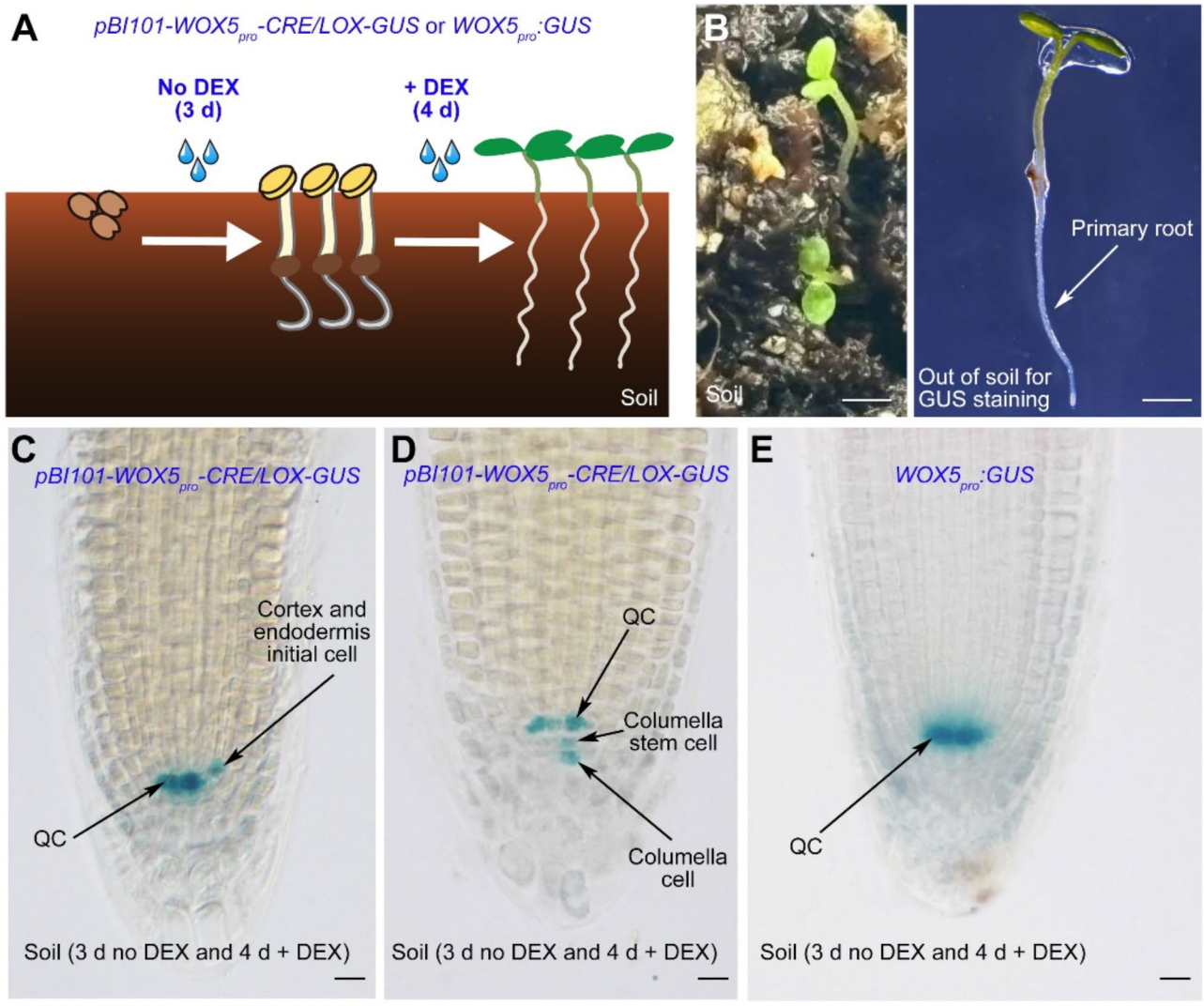

Fig. 4 Cell lineage in the stem cell niche of RAM in soil. **A**, **B**. Diagram (A) and pictures (B) of seedlings grown in soil. Seeds were germinated and seedlings were grown in DEX-free soil (no DEX) for 3 days (d), then watered every 12 h with an aqueous solution of 100 μM DEX (+ DEX) for 4 days (C–E). **C**, **D**. GUS staining of the root of the 7-day-old *pBI101-*$WOX5_{pro}$*-CRE/LOX-GUS* transgenic line grown in soil with DEX treatment. Note that GUS signals were present in cortex and endodermis initial cells (C), columella stem cells (D), and columella cells (D) in soil. A total of 19 roots were observed, and 8 of them showed GUS signals in initial/stem cells or columella cells in addition to the QC cells. **E**. GUS staining of the root of the 5-day-old $WOX5_{pro}$:*GUS* transgenic line grown in soil. A total of 19 roots were observed, and all of them showed GUS signals in the QC cells. Two independent lines were analyzed and showed the same results. Scale bars, 1 mm in (B) and 50 μm in (C–E)

we cultured the hypocotyls of the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* line on CIM containing DEX for 5 days and 30 min (constant DEX treatment) to form callus, and then the callus was cultured on SIM without DEX for 7 days (Fig. 5A). In the second condition, we cultured the hypocotyls of the *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* line on CIM without DEX for 5 days and then with DEX for 30 min (transient DEX treatment) to form callus, and then the callus was cultured on SIM without DEX for 7 days (Fig. 5C).

In the first condition (constant DEX treatment), the CRE protein had sufficient time for *LOX2272* recombination in almost all cells in the middle cell layer. Consequently, GUS signals were observed in almost all cells in the regenerated SAM (Fig. 5A and B), consistent with previous observations using the two-construct CRE/LOX system [16].

In the second condition with transient DEX treatment on CIM, the CRE protein had limited time for *LOX2272* recombination in some of the cells in the middle cell layer, resulting in a mosaic of positive and negative GUS signals in the regenerated SAM (Fig. 5C and D). This result indicates that the SAM arises not from a single shoot progenitor cell, but from a group of shoot progenitor cells on SIM.

All-in-one *pBI101-CRE/LOX-RUBY* system

To trace the cell lineage in live organs in a real-time and in vivo manner, we constructed the *pBI101-CRE/LOX-RUBY* system (Fig. 6A and B) by replacing the *GUS* gene in *pBI101-CRE/LOX-GUS* with *RUBY*, which encodes the enzymes that convert tyrosine to red betalain [33]. Betalain is visible to the naked eye without further staining treatments.

To test this system, we cloned the *WOX11* promoter into *pBI101-CRE/LOX-RUBY*, generating *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY*. The *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY* construct was transformed into wild-type *Arabidopsis* Col-0. The red betalain signal was clearly observed in adventitious roots regenerating from detached leaves of the *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY* line (Fig. 6C and D), supporting the previous finding that adventitious roots originate from *WOX11*-marked adventitious root founder cells [6].

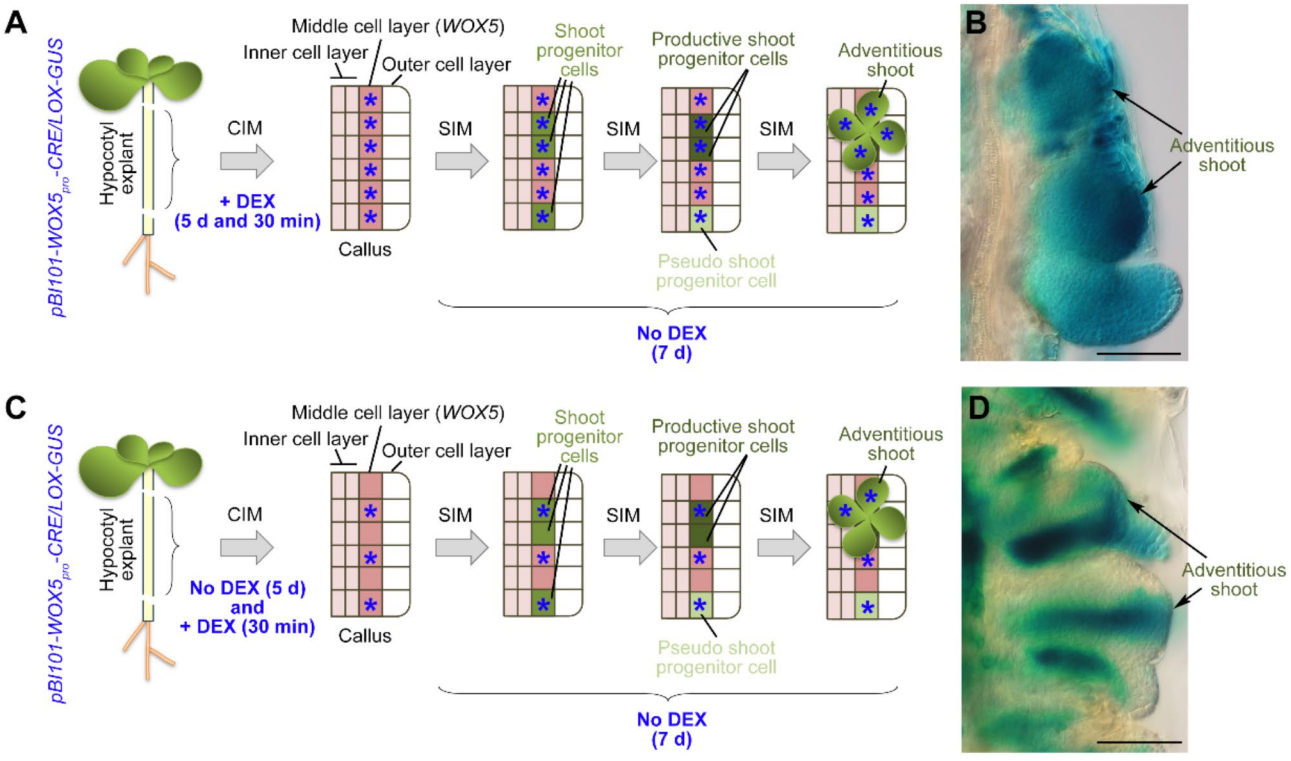

Fig. 5 Cell lineage during SAM formation in tissue culture. **A**, **B**. Diagram (A) and GUS staining result (B) of SAM regeneration in tissue culture with constant DEX treatment on CIM. Hypocotyl explants from *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* transgenic line were cultured on CIM with 10 μM DEX (+DEX) for 5 days and 30 min to form callus, which was then moved to SIM without DEX (no DEX) for 7 days. **C**, **D**. Diagram (C) and GUS staining result (D) of SAM regeneration in tissue culture with transient DEX treatment on CIM. Hypocotyl explants from *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* transgenic line were cultured on CIM for 5 days without DEX, moved to CIM with 10 μM DEX for 30 min, then callus was moved to SIM without DEX (no DEX) and cultured for 7 days. Blue asterisks indicate cells with *LOX2272* recombination event, showing positive GUS staining signals (A, C). Two independent lines were analyzed and showed the same results. Scale bars, 100 μm in (B, D)

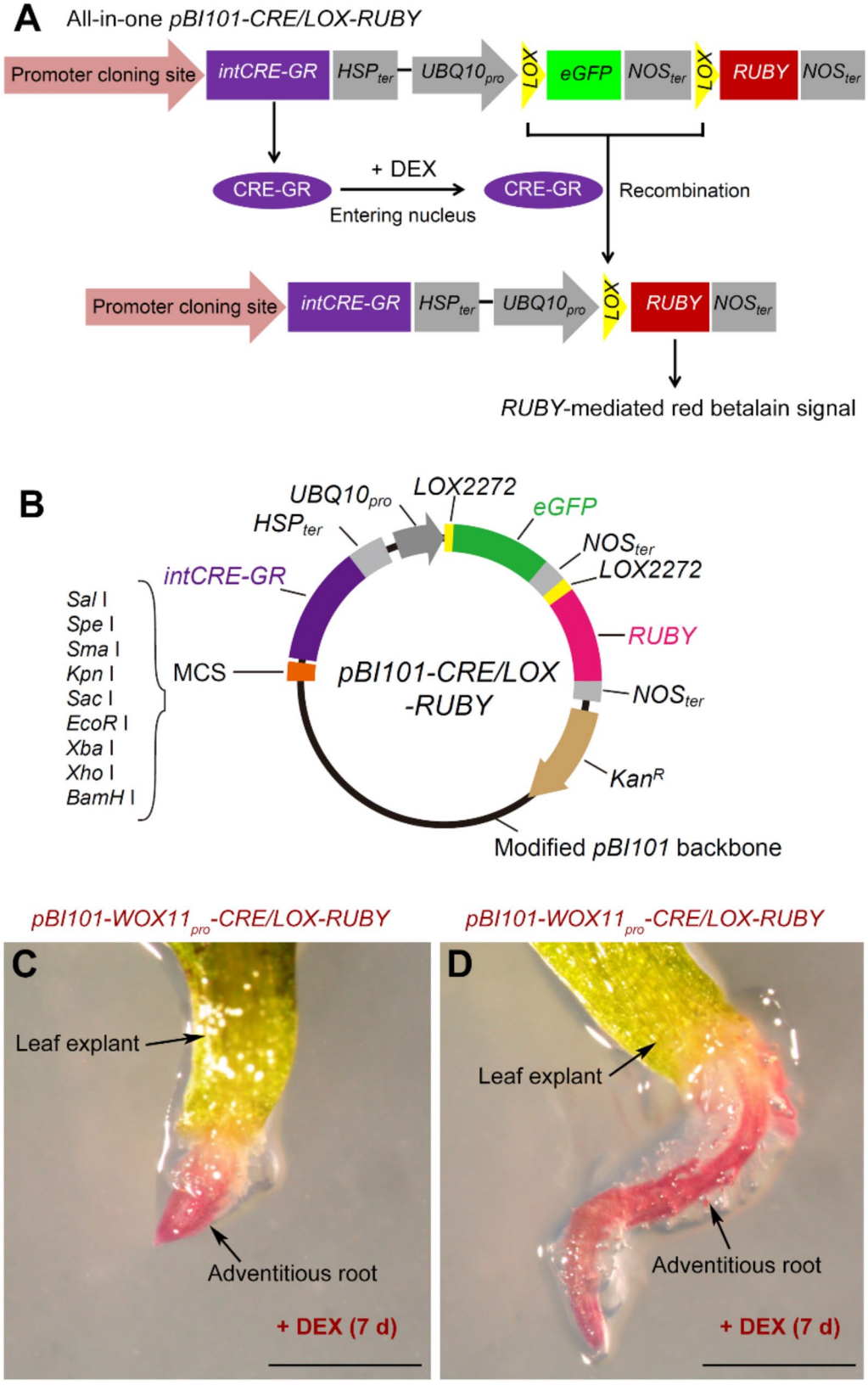

Fig. 6 (See legend on next page.)

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Fig. 6 The *pBI101-CRE/LOX-RUBY* system. **A**. Schematic of the all-in-one *CRE/LOX-RUBY* system to trace cell lineage in vivo. **B**. Schematic of plasmid vector *pBI101-CRE/LOX-RUBY*. **C**, **D**. Images of the *pBI101-WOX11*$_{pro}$*-CRE/LOX-RUBY* transgenic line, showing positive red betalain signal in adventitious roots developed from detached leaves. Seedlings were germinated on DEX-free ½ MS medium, grown for 12 days, and first-pair leaves were detached and cultured on B5 medium with 10 μM DEX treatment (+DEX) for 7 days. Two independent lines were analyzed and showed the same results. Scale bars, 1 mm in (C, D)

Discussion

In this study, we describe the all-in-one CRE/LOX system that is able to trace cell lineage based on GUS signals or *RUBY*-mediated red betalain signals.

Using this system, we found that stress conditions might have effects on the cell lineage or cell identity in the RAM. The *pBI101-WOX5*$_{pro}$*-CRE/LOX-GUS* lines showed positive GUS signals in the initial/stem cells in addition to the QC when the roots were under osmotic stress conditions or grown in soil. It is possible that the QC cells within the RAM divide and replenish initial/stem cells when the root is grown under stress conditions or in soil. Our previous study also indicated that the QC contributes to the root cap cell lineage and the vascular cell lineage when the plant was vertically grown on ½ MS medium for a long time [29]. It was proposed that the QC might serve as a cell reservoir for reconstituting damaged cells within the meristem, and QC could exhibit active cell division in response to damage in the RAM [39–43]. It will be important to comprehensively analyze the RAM development mechanism and cell lineages in the RAM in roots growing under stress conditions and in soil in the future.

Previous studies have proposed that the SAM might beformed from multiple cells in plant materials cultured on SIM in tissue culture [24, 44]. Our data support this idea, because we observed that the SAM formed from a group of shoot progenitor cells, rather than a single shoot progenitor cell. It is important to note that during *Agrobacterium*-mediated gene transformation or gene editing, the regenerated plant might be a mosaic if the *Agrobacterium* is added after materials have been cultured on CIM or SIM for some time. It is not clear why only groups of shoot progenitor cells are able to establish the fate of productive shoot progenitor cells, which can further proliferate into a SAM. One possibility is that the formation of heterogeneous gene expression patterns within the shoot progenitor cell group is essential for the fate establishment of the productive shoot progenitor cells, leading to the SAM formation [24]. In further studies, it will be interesting to study the mechanism by which shoot progenitor cells collaboratively give rise to the productive shoot progenitor cells in plant tissue culture on SIM.

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Author contributions
C.L., L.X., N.Z. conceived the study. S.W., Y.L., Z.L., and N.Z. performed experiments. L.X. and N.Z. wrote the manuscript. All authors discussed and analyzed the data.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors agree with the submission of this manuscript to Plant Methods.

Competing interests
The authors declare no competing interests.

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