Research Article

Poly (ADP-ribose) polymerases regulate cell division and development in *Arabidopsis* roots

Running title: PARPs regulate root development

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ABSTRACT

Root organogenesis involves cell division, differentiation and expansion. The molecular mechanisms regulating root development are not fully understood. In this study, we identified poly (ADP-ribose) polymerases (PARPs) as new players in root development. PARP catalyzes poly (ADP-ribosyl)ation of proteins by repeatedly adding ADP-ribose units onto proteins using nicotinamide adenine dinucleotide (NAD^+) as the donor. We found that inhibition of PARP activities by 3-aminobenzomide (3-AB) increased the growth rates of both primary and lateral roots, leading to a more developed root system. The double mutant of *Arabidopsis* PARPs, *parp1parp2,* showed more rapid primary and lateral root growth. Cyclin genes regulating G1-to-S and G2-to-M transition were up-regulated upon treatment by 3-AB. The proportion of 2C cells increased while cells with higher DNA ploidy cells declined in the roots of treated plants, resulting in an enlarged rootmeristematic zone. The expression level of *PARP2* was very low in the meristematic zone but high in the maturation zones, consistent with a role of PARP in inhibiting mitosis and promoting cell differentiation. Our results suggest that PARPs play an important rolein root development by negatively regulating root cell division.

Keywords *Arabidopsis thaliana*, cell division, inhibitor, poly (ADP-ribose) polymerase, protein activity, root development

INTRODUCTION

Plant roots take up water and nutrients from the soil for growth and development (Osmont et al. 2007; Lucas et al. 2008). Dicotyledonous plants generally have a tap root system, with the primary root (PR) growing down into soil and lateral roots (LR) growing horizontally outward from the primary root (Hochholdinger et al. 2004). Root architecture is an important aspect of plant growth and plays an important role in the plant's ability to adapt to adverse environments (Hochholdinger et al. 2004; Osmont et al. 2007). Plants with well developed root systems usually have higher biomass yield and better resistance to stress (Malamy and Ryan 2001; Hodge 2006). Therefore, studying the molecular mechanisms controlling plant root development is important for agriculture.

The PR is formed embryonically while LRs are generated postembryonically. LR development can be divided into three major steps: pre-initiation, initiation and post-initiation (Malamy and Ryan 2001; Hodge 2006). Auxin is considered to play an important role in the whole process of LR development (Beeckman et al. 2001; Casimiro et al. 2003; Peret et al. 2009). LR initiates from the pericycle founder cells in the PR. Auxin down-regulates the expression of CDK inhibitor *KRP2* (*KIP-RELATED PROTEIN 2*) at the sites where LR primordium will form, promotingre-entry into mitosis of the xylem-pole pericycle cells which establishes the LR founder cells(Casimiro et al. 2003). Through multiple rounds of asymmetric cell divisions of the founder cells(Malamy and Benfey 1997), a dome-shaped LR primordium is formed. The primordium penetrates thorough the endodermis, cortex and epidermis by cell division and expansion, and emerges from the PR in the differentiation zone (Malamy and Benfey 1997;

Casimiro et al. 2003). After emergence, the LR apical meristem is activated and the LR elongates quickly. The LR then starts to show features similar to the PR (Petricka et al. 2012).

Along their longitudinal axes, both PRs and LRs can be divided into four different developmental zones: root cap, meristematic zone, elongation zone and differentiation (maturation) zone (Scheres et al. 2002; Petricka et al. 2012). Root growth is sustained by the stem cells in the meristematic zone. Cells close to the boundary between the meristematic zone and the elongation zone exit the mitotic cycle and switch into the endoreplication cycle (endocycle), giving rise to cells with nuclear DNA content of 4C, 8C, 16C or 32C due to repeated DNA replication without cytokinesis (Galbraith et al. 1991). Meanwhile, these cells undergo rapid elongation and expansion, leading to root growth. The boundary between the meristematic zone and elongation zones determines the meristem size, which is directly correlated to the root growth rate (Lucas et al. 2008; Petricka et al. 2012; Hayashi et al. 2013)**.** In the proximal differentiation zone of the root, cells differentiate into various cell layers, such as the epidermis, cortex, endodermis, pericycle and vasculature (Petricka et al. 2012). Cell division and cell differentiation maintain a dynamic balance in normal developing roots and therefore are important processes for root development. Exiting the mitotic cycle and undergoing endoreplication is the starting point of cell differentiation (De Veylder et al. 2003; Polyn et al. 2015). Many genes or proteinshave been identified to function in this process. For example, CDC27B, one of the components of anaphase-promoting complex/cyclosome (APC/C) complex (Blilou et al. 2002; Perez-Perez et al. 2008), *HIGH PLOIDY2* (*HPY2*) along with

PLT genes(Ishida et al. 2009), and a small ubiquitin-like modifier (SUMO) E3 ligase prevent endocycle onset in meristematic zone. The *PLT* genes act through *HPY2* to promote the mitotic cell cycle (Galinha et al. 2007; Ishida et al. 2009). The APC/C activity controller CCS52A1, plays an opposing role in this process (Vanstraelen et al. 2009). Despite theseprogress, the switching mechanism between mitosis and endocycling is only partially understood.

PARPs (Poly(ADP-ribose) polymerases) are one type of enzyme which can repeatedly catalyze the transfer of an ADP-ribose moiety from NAD^+ onto protein substrates in the presence of broken DNA, thus leading to the post-translational modification of target proteins.They have been found to regulate many biological processes such as DNA repair, gene transcription, epigenetic regulation, stress response and cell death in animal systems (Gibson and Kraus 2012; Kalisch et al. 2012; Luo and Kraus 2012; Swindall et al. 2013; Leung 2014). PARP inhibitors, such as 3-aminobenzamide (3-AB), benzamide (BA) and 3-methoxybenzamide (3MB), have been used for PARP activity inhibition in both animals and plants (Virag and Szabo 2002; De Block et al. 2005; Adams-Phillips et al. 2010; Rouleau et al. 2010; Schulz et al. 2012; Schulz et al. 2014). There are three PARPs in *Arabidopsis* but only two of them, PARP1 and PARP2, have been reported to be active PARPs (Babiychuk et al. 1998; De Block et al. 2005), while the catalytic activity of PARP3 has not yet been demonstrated. In mouse, the *parp1-/-/parp2-/-* double mutant is embryo lethal, indicating an essential role for PARPs in embryogenesis (Menissier de Murcia et al. 2003). In recent years, the functions of PARPs in *Arabidopsis* have been studied through mutant characterization or pharmaceutical inhibition. PARPs are involved in DNA repair,

plant growth, seed germination, abiotic and biotic stress tolerance (De Block et al. 2005; Vanderauwera et al. 2007; Ishikawa et al. 2009; Schulz et al. 2012; Jia et al. 2013; Liu et al. 2014; Rissel et al. 2014; Schulz et al. 2014; Feng et al. 2015; Pham et al. 2015; Song et al. 2015; Zhang et al. 2015). So far, whether and how PARPs regulate root development has not been addressed.In this study, we reportthat *Arabidopsis* PARPs negatively regulate root cell division and that inhibition of PARPs results in a more developed root system. We further show that the involvement of PARPs in cell division is independentofthe auxin signaling pathway.

RESULTS

3-AB inhibits the activities of PARP1 and PARP2 *in vitro* and *in vivo*

3-AB is used in animal for pharmaceutical purposes and its effects on animals have been widely examined (Virag and Szabo 2002; Adams-Phillips et al. 2010). However, its mode of action on plant PARPs has not been fully illustrated. Thus, we tested its effects on the two active *Arabidopsis* PARPs, PARP1 and PARP2, *in vitro* and *in vivo*. Poly(ADP-ribose) polymerase can attach ADP-ribose polymers (PAR) to itself and the auto-modification activity is often used to determine its activity (Langelier et al. 2008; Altmeyer et al. 2009). We expressed the full-length *Arabidopsis* PARP1 and PARP2 in *E.coli*. The purified PARP1 and PARP2 recombinant proteins were incubated with NAD^+ for different time periods in the presence of broken double-strand DNA in a reaction buffer. PARP1 and PARP2 both generated PAR in a time-dependent manner, with the protein band shifting upward due to the addition of PAR molecules. The PAR antibody recognized only long ADP-ribose polymers on proteins because of the

weak immunogenicity of short polymers. 3-AB inhibited the activities of both PARP1 and PARP2 (Figure 1A, B). We also extracted the total proteins from *Arabidopsis* roots and examined *in vivo* PAR level by dot blot. The double-strand DNA break inducing agent zeocin stimulated the synthesis of PAR while 3-AB inhibited the accumulation of PAR *in vivo* (Figure 1C). We further used anti-PARP2 antibody to detect the mobility shift of PARP2 protein in *Arabidopsis* seedling extracts. The up-shifted smears originating from the self-modification of PARP2 protein were weakened by co-incubation of 3-AB (Figure 1D). Taken together, this indicates that 3-AB inhibited PARP activities *in vitro* and *in vivo*.

Arabidopsis plants treated with PARP inhibitors have a more developed root system

To observe the growth phenotype when PARP activities are inhibited, we germinated *Arabidopsis* seeds on 1/2 MS plates including 3-AB. We found that *Arabidopsis* plants grown on 3-AB plate had much more developed root systems than those grown without 3-AB (Figure 2A, B). The plants treated with 3-AB exhibited more lateral roots than control plants and the long lateral roots branched further to produce secondary lateral roots, leading to a more complicated root architecture (Figure 2B, C). The seedlings grown with 3-AB also had a higher average biomass than those without (Figure 2D).

To observe the lateral root development process more clearly, we grew the plants vertically on the surface of 1/2 MS plates with or without 3-AB. The root development of *Arabidopsis* seedlings was analyzed from the 6^{th} day to 14^{th} day after germination, and only lateral roots emerging from the primary root were counted. During this period, no secondary lateral roots were observed. We again

noticed that seedlings treated with 3-AB had more lateral roots than control plants in average (Figure 2E), and these lateral roots grew faster than those of control (Figure 2F), resulting in a significant increase in total lateral root length of 3-AB treated plant (Figure 2G).

When another PARP inhibitor 6(5H)-phenanthridinone (Virag and Szabo 2002) was used to perform the same experiment, it also had a similar effect on the root growth, although weaker than 3-AB (Figure S1).

To determine which step(s) of LR development is/are affected by PARP inhibitors, the auxin reporter line, *DR5::GUS*transgenic plant(Ulmasov et al. 1997)was used to visualize the LR primordia embedded in PR by GUS staining (De Smet et al. 2007). On the 10^{th} day after germination, 3-AB treated plants had 2 to 3 visible LRs in the basal part of the PR, while fewer and shorter LRs were observed in the non-treated control plants (Figure 3A). The PR of the 3-AB treated plants was also longer than that of control plants (Figure 3B). If the embedded LR primordia and emerged LRs were counted together, no significant difference was observed between the control and the treated plants (Figure 3C). We also used the promoter-GUS reporter line, *CycB1;1::GUS* plants (Ferreira et al. 1994) to observe the LR development process, similar results were obtained (Figure S2). These results showed that inhibition of PARP activities mainly promoted LR development after LR initiation, instead of LR initiation, since the total LR numbers were not changed. The LRs of 3-AB treated plants grew faster than those of control plants, which might produce secondary lateral roots again and increased the lateral root numbers.

Inhibition of PARP activities enhances root cell division

Root growth involves cell division in the meristematic zone and cell elongation in the elongation zone (Veylder et al. 2007). The meristematic cells generate diploid daughter cells through active mitosis while the cells in the elongation zone undergo endoreplication giving rise to cells with DNA ploidies larger than 2C. To observe if inhibition of PARPs expedites the root growth via promoting mitotic cell cycle, the DNA ploidy of the roots grown with PARP inhibitor treatment was analyzed and compared with that of control plants. The results showed that the ratio of 2C cells increased while those of higher DNA ploidies, such as 8C and 16C decreased in the 3-AB treated plants (Figure 4A). It suggested thatinhibition of PARP activity enhanced mitotic cell division, while repressing endoreplication in roots.

CDK (Cyclin-dependent kinase)-cyclin complexes are important cell cycle regulators. The G1-to-S transition is primarily regulated by the association of CDKA with D-type cyclins (CYCDs) (Mironov et al. 1999; Inze and De Veylder 2006). KRP2 inhibits the G1-to-S phase transition by interacting with CDKA and CYCD (De Veylder et al. 2007; Sanz et al. 2011). B-type cyclins associate with Cdc2 kinases and assist in the G2-to-M transition (Mironov et al. 1999; Potuschak and Doerner 2001).

To study the impact of PARP inhibition of the cell cycle in roots, the expression levels of cell cycle controlling genes were examined by quantitative reverse transcription PCR analysis (qRT-PCR) using seedlings sprayed with 1mM 3-AB. As shown in Figure 4B, the expression of mitotic regulators, such as B-type cyclins (*CycB1;1* and *CycB1;4*) and D-type cyclins (*CycD2;1* and *CycD3;3*),

were strongly induced in the roots of *Arabidopsis* treated by 3-AB. The expression level of *KRP2*, which inhibits lateral root development in *Arabidopsis*, was reduced. In the aerial part of the plants, these genes did not show the same changes as they did in roots. By propidium iodide(PI) staining, the root meristem size of *Arabidopsis* plants grown on 3-AB plates was compared with that of control plants. The results showed that 3-AB treated plants had a longer root meristematic zone, consistent with the observation that the roots of 3-AB treated plants grew faster than those of control plants (Figure 4C, D). These results suggested that the PARP inhibitor 3-AB promoted cell cycle progression in roots at both the G1 to S and G2 to M phase transitions. Thus, the activation of PARP activities blocks cell cycle progression.

3-AB functions independently of auxin signaling

Auxin is known as a positive factor regulating *Arabidopsis* lateral root initiation and elongation (De Smet et al. 2007). IAA at low concentrations promotes primary and lateral root growth (Mulkey et al. 1982), while high concentrations of IAA inhibits primary root elongation and lateral root formation (Eliasson et al. 1989; Poupart et al. 2005). We therefore tested the possible interplay between the auxin signaling pathway and the 3-AB affected pathway. The experiments were performed with 1 nM (low concentration) and 1 μM IAA (high concentration), respectively. 1 nM IAA promoted primary root elongation and also increased the lateral root number (Figure 5A-C). When 1 mM 3-AB was added together with 1 nM IAA, the effects on root growth werestill notable (Figure 5B, C). 3-AB further enhanced root growth and increased lateral root numbers. In contrast, 1 μM IAA

inhibited root development. 3-AB still stimulated primary root growth and increased lateral root numbers of IAA-inhibited plants (Figure 5B, C). These results suggested that 3-AB acts independently ofthe auxin signaling pathway. However, since auxin plays an overall regulatory role in plant growth, the final effects of 3-AB on root growth may still be influenced by IAA to some degree, as shown by the two-way ANOVA analysis results (ReportsS1, S2).

To further analyze the process that 3-AB is involved in, we used the *aux1* mutant to test the effect of 3-AB. AUX1 encodes an auxin permease which mediates phloem-based IAA influx transport (Bennett et al. 1996; Swarup et al. 2001). The *aux1* mutant has fewer lateral roots, and shows defective root gravitropism. Our results revealed that, 3-AB also increased the emerged lateral root number of the *aux1* mutant, but it was still less than that of wild type plants (Figure 5D). 3-AB did not recover the root gravitropism defect of the *aux1* mutant (Figure S3), further indicating that 3-AB does not function in root development process through auxin transport and signaling.

We also used adouble mutant of auxin signaling pathway, *arf7arf19* to observe the effect of 3-AB. LR initiation is impaired in the *arf7arf9*mutant because ARF7/ARF19 proteins are necessary for activating the downstream genes important for initiating LR development(Okushima et al. 2007). We found that 3-AB failed to induce more LRs in this double mutant (Figure 5D), consistent with the previous observation that 3-AB did not promote new LR initiation (Figure 3C). These results togethersuggest that 3-AB functionsdownstream of LR initiation.

The *parp1parp2* double mutant exhibits similar root development phenotypeto 3-AB treated plants

To understand if the root growth phenotype of 3-AB-treated plants is caused by the disruption of PARP activities, we studied the phenotype of the *Arabidopsisparp1parp2* double mutant. The double mutantshowed more lateral roots and longer primary root than wild type Col-0 plants when grown on standard 1/2 MS plate (Figure 6A-C). However, the phenotype was weaker than that seen with PARP inhibitors. The stimulating effect of 3-AB on lateral root development on *parp1parp2*plants was not as obvious as that on Col-0 plants-.

In addition, we observed the phenotype of the *parp1parp2* mutant grown on IAA plates. Figure S4B gives the comparison of the phenotypes of Col-0 and the *parp1parp2* mutant grown for 22 days, because no difference was observed when the plants had grown for two weeks. The double mutant responded similarly to IAA treatments as wild type Col-0 plants at both high concentration and low concentration of IAA(Figure S4), further supporting that PARPs participate in a pathway different from the auxin signaling pathway.

***PARP2*and *PARP1* are expressed in roots**

To observe the expression patterns of *PARP1* and *PARP2* in roots, we obtained *proPARP1::GUS* and *proPARP2::GUS* transgenic lines. GUS staining results showed that *PARP2* was expressed at a higher level in root differentiation zone than in the meristematic zone (Figure 7A, B), consistent with a repressive role of PARP2 in cell division. The expression of *PARP2*was seen in the elongation zone of young lateral roots just after emergence, but very low in the meristematic

zone of LRs (Figure 7C, D). GUS signal was not detected in the roots of*proPARP1::GUS* transgenic *Arabidopsis* during the whole root developmental process, probably due to the low expression of *PARP1* in the roots. qRT-PCR results showed that *PARP1* was expressed in roots, although lower than that of *PARP2* (Figure 7E).

PARP1 interacts with PARP2 *in vitro* and *in vivo*

In animals, it has been reported that PARP1 can form heterodimers with PARP2 (Schreiber et al. 2002). In *Arabidopsis*, PARP1 and PARP2 are both localized in nucleus (Babiychuk et al. 1998). To test whether they interact with each other,we used a yeast two-hybrid assay. The results showed that PARP1 interacted with PARP2 in yeast cells (Figure S5). Bimolecular fluorescence complementation (BiFC) experiments indicated that PARP1 interacted with PARP2 *in planta* (Figure S3B). A recent study (Song et al. 2015) also indicated that *Arabidopsis* PARP1 and PARP2 interact with each other *in vivo* in regulating the plant immune response.

DISCUSSION

Primary and lateral root developmental processes determine the root architecture of dicotyledonous plants. The postembryonic development of the primary root is mainly determined bycell division in the meristematic zone and cell differentiation in the elongation zone. Lateral rootsare initiated in the elongation zone but emerge in the maturation zone (Casimiro et al. 2003; Petricka et al. 2012).Many genes have been identified to be involved in both primary and lateral root development, and most of them are related to hormone

signaling such as auxin. Players involved in root development but independent of auxin signaling are less known. In this study, we identified *PARPs* as players in root development through the regulationof cell division.

Our results showed that 3-AB treated plants and the *parp1parp2* double mutant had similar responses to IAA treatments as Col-0. On the other hand, the auxin signaling mutant *aux1*, also responded to 3-AB similarly as Col-0. These results indicate that the effect of PARP inhibitor on root development is not achieved through auxin signaling. 3-AB failed to stimulate lateral root initiation in *arf7arf19* double mutant, suggesting that 3-AB functions after LR initiation.

Previous studies reported that the inhibition of PARPs increases plant biomass production and also leaf cell numbers (Schulz et al. 2012; Schulz et al. 2014). When we examined the expression patterns of PARPs under normal conditions, we found that *PARP1* and *PARP2* are predominantly expressed in the developing roots in the young seedlings. PARPs have been reported to mainly mediate DNA repair and cell death in both animal and plant systems (Luo and Kraus 2012; Swindall et al. 2013; Leung 2014; Zhang et al. 2015). They are involved in embryogenesis and neurogenesis in *Drosophila* and mouse, and knock-out mutants of *PARPs* display an embryo lethal phenotype (Menissier de Murcia et al. 2003; Hanai et al. 2004; Koh et al. 2004). However, all *parp* mutants in *Arabidopsis* are viable and morphologically normal, indicating a big difference in function between animal and plant PARPs. A working model of *ArabidopsisPARPs* in root development was proposed based on our results (Figure S6). PARP1 and PARP2 interact with each other and inhibit root cell division. When their activities are inhibited by 3-AB, the root mitosis is enhanced

and leads to a more developed root system.

During development, DNA repair is activated when the genome is damaged by internal or external factors (Cools and De Veylder 2009). PARP can detect single-or double-strand DNA breaks and responds by self-activation. It catalyzes PAR synthesis on itself and then recruits proteins responsible for repairing DNA damages by PAR chains (Schreiber et al. 2002). Therefore, DNA repair is initiated and cell cycle progression is temporally halted. In our study, PARP2 is more abundantly expressed in the differentiation zone but is low in the meristematic zone. PAR may act as a signal to arrest the cell cycle in the zones without mitosis and thereby promote differentiation. It is reported that over-expression of poly(ADP-ribose) polymerase promotes cell cycle arrest of NB4 acute promyelocytic leukemia cells(Bhatia et al. 1996).

In murine embryonic fibroblasts, PARP-1 modifies ATM and ATR kinases and PARP activity is required for optimal ATM- and ATR-mediated DNA repair signaling pathways (Aguilar-Quesada et al. 2007; Horton et al. 2007; Kedar et al. 2008). ATM and ATR kinases regulate cell cycle checkpoint activation, which slows or arrests cell cycle progression for correcting genetic errors before they are passed on to the daughter cells (Abraham 2001). Interaction between PARP-1 and ATR in mouse fibroblastsis is disrupted by inhibition of PARP (Kedar et al. 2008), which affects the DNA repair-regulated cell cycle progression. In plants, ATM and ATR also mediate the conversion from mitosis to endocycle in addition to DNA repair (Adachi et al. 2011; Sherman et al. 2011; Yoshiyama et al. 2013). It is likely that inhibition of PARP activities blocks the

activation of ATM and ATR kinases, therefore impedes the entrance to endocycle but enhances root cell division in Arabidopsis.

Inhibition of PARPs resulted in a larger root meristem, higher proportion of 2C cells and lower proportions of polyploid cells in the root cell populations. These data further support that cell division was enhanced while endoreplication was relatively repressed. Up-regulation of cyclin genes also indicated that mitosis is enhanced in the roots. PARPs might play a role in the switch between cell division and cell differentiation in the roots. Genes controlling this switch, such as *HPY2*, *CCS52A1* (Ishida et al. 2009; Vanstraelen et al. 2009), have different expression patterns in the meristematic zone and other zones. *PARP2* is also expressed differently in the meristematic zone and differentiation zone, consistent with a possible role of PARP in the cell fate determination between cell division and cell differentiation.

The *parp1parp2* double mutant has longer primary root and more lateral roots than wild type plants, supporting that interruption of PARP function indeed contributed to the phenotype observed with PARP inhibitors. However, the phenotype caused by 3-AB is stronger than that of *parp1parp2* double mutant. It is possible that PARP inhibitor may also inhibit the activities of other potential PARPs, such as PARP3 or the members of RCD-SRO family, which also carry a core PARP catalytic domain, but no biochemistry activity has been demonstrated so far (Jaspers et al. 2010).

It has been shown by Pham et al. (2015) that the single mutants of *PARP* family members have longer primary roots than wild type in the early stage of

seedling development. We failed to see this phenotype for *parp1* and *parp2* single mutants, probably due to the subtle difference of culture conditions used in different labs, or the weak phenotype. However, we did observe that the *parp1parp2* double mutant exhibited more rapid lateral root growth than Col-0 at the same developmental stage, which has not been reported before. PARP inhibitors, such as 3-AB, can be used for chemical inhibition of all active PARPs to enhance root development.Our results illustrated a role for PARPs in *Arabidopsis* root development by regulating root cell division, and also implied the potential value of PARP inhibitors in improving plant root architecture.

MATERIALS AND METHODS

Plant materials and growth conditions

Arabidopsis materials used in this study are of the Columbia ecotype (*Arabidopsis thaliana* L. *cv. Columbia*). Seeds were sown on 1/2 Murashige-Skoog (MS) plates, stored for 3 days at 4°C, and then grown at 22°C under long-daylight condition (16 h light/8h dark). For inhibition of PARP activity, 1 mM3-AB was included in the media. All plants were grown on plates vertically placed unless otherwise stated.

Root Growth Analysis

Arabidopsis seedlings were grown on control plates or on 1 mM 3-AB plates, respectively. To track changes of lateral root growth, the seeds were placed on plate more dispersed than usual. The root growth status were monitored daily

from 6 DAG (day after germination) to 14 DAG. Several indexes were recorded each day: LR number, number of emerged LRs; LR length, the length of emerged LR (>1mm) was measured every day. LR length is the total value of all lateral root lengths; LR elongation rate, the length changes of LRs longer than 3 mm were recorded every day. The total LR length changes were divided by LR number to give the LR elongation rate.

GUS activity assay

The 1,328 bp promoter fragment upstream of the start codon of *PARP1* gene and 2,180 bp fragment upstream of the *PARP2* gene were cloned into the pAKK vector which carries a β-glucuronidase (GUS) reporter gene. These recombinant plasmids were used for *Agrobacterium* transformation and then plant transformation.

PARP1::GUS, PARP2::GUS, DR5::GUS and CycB1;1::GUS transgenic lines were grown vertically on 1/2 MS plates with or without 1 mM 3-AB. The seedlings were collected and treated with 90% cold acetone for 10 min, and then stained in 50 mM pH 7.0 sodium phosphate buffer containing 2 mM $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, 0.1% (v/v)Triton X-100 and 1 mg/mL X-GLUC at 37°C overnight. Absolute ethanol was used to destain the tissues before photographing.

DNA ploidy analysis

Arabidopsis were grown vertically on 1/2 MS plates with or without 1 mM 3-AB for one week. Around 100 roots for each sample were collected and chopped

with new razor blades in 5 mL buffer (10 mM Mg_2SO_4, 50 mM KCl, 5 mM HEPES, pH 8.0, 0.25% Triton X-100 and 6.5 mM DTT) to release the nuclei, and then filtered through a 100 μm mesh twice. Propidium iodide (PI) was added to the nucleus preparation at a final concentration of 50 μg/mL. The samples were then analyzed with the BD FACSCalibur™ system (BD Biosciences, USA).

Quantitative reverse transcription PCR (qRT-PCR)

One-week-old *Arabidopsis* seedlings were sprayed with1 mM 3-AB or with water for controluntil the leaves were fully wet and then incubated in growth chamber under normal conditions for 24 h. The aerial parts and roots of the seedlings were collected respectively and quickly frozen in liquid nitrogen. Total RNA was extracted using Trizol reagent according to the manufacturer's instructions (TaKaRa, Japan). 1 μg total RNA was used for cDNA synthesis using Takara PrimerScipt RT reagent kit with gDNA Eraser (TaKaRa, Japan). The reactions were performed with SYBR Green QPCR Master Mix (Takara, Japan) in a Real One Plus Real-Time PCR System (Applied Biosystem, USA). Each sample was analyzed in triplicate. Primers used are listed inTable S1.

Expression and purification of recombinant proteins

The cDNA fragments of *PARP1* and *PARP2* were cloned into *pET32a* vector (Novagen, USA) in fusion with N-terminal hexahistidine and thioredoxin tags. The recombinant vectors were transformed into the *Escherichia coli* host strainRosetta (DE3). Protein expression was induced with 100 μM isopropyl β-D-thiogalactoside at 16°Cfor 20 h. The recombinant PARP1 and PARP2

proteins were purified with HisPur Ni-NTA resin (Thermo Scientific, USA) based on the manufacturer's manual.

DNA-dependent auto-modification assay

Enzymatic activities of recombinant PARP1 and PARP2 proteins were analyzed as described (Altmeyer et al. 2009) . To detect the effect of 3-AB on the activities of PARP1 and PARP2, 3-AB was added into the poly(ADP-ribosyl)ation reaction mixtures to a final concentration of 1 mM. Reactions were stopped by addition of SDS-PAGE loading buffer and boiled for 5 min at 95°C before loaded onto SDS-PAGE gel.

Yeast two-hybrid analysis

Yeast two-hybrid analysis was performed using the Matchmaker Gold Y2H system (Clontech, USA). cDNAs of PARP1 and PARP2 were cloned into *pGADT7* and *pGBKT7* vectors and then introduced into the yeast strain Y187 and Y2H gold, respectively by the lithium acetate method (James et al. 1996). The two strains were then mated based on the protocol provided by the manufacturer. The mated colonies were initially grown on media lacking tryptophan and leucine (–Trp/-Leu), and then streaked onto selective medium lacking tryptophan, leucine, histidine and adenine (-Leu/-Trp/-His/-Ade) without 3-AT. Interactions between the co-expressed proteins were assessed by the growth of yeast transformants on the SD/-Leu/-Trp/-His/-Ade media and also by β-galactosidase filter assay following the manufacturer manual.

Bimolecular fluorescence complementation (BiFC) assay

cDNA fragments of *PARP1* and *PARP2* were cloned into YFP^N (*pXY103*) and YFP^C (*pXY104*) vectors(Bracha-Drori et al. 2004), respectively. The agrobacteria carrying *PARP1-YFP*^{N} and *PARP2-YFP*^{C} vectors were mixed at a ratio of 1:1, and then used for tobacco leaf infiltration. For positive control of fluorescence, agrobacteria containing the empty vector *35S::YFP* was used. For negative control, mixture of agrobacteria containing empty YFP^N (YFP^C) vector and *PARP2-YFP*^{C} (*PARP1-YFP*^{N}) were used. Two days later, YFP fluorescence was monitored using a Zeiss model LSM510 confocal microscope (Carl Zeiss, Germany).

Propidium iodide (PI) staining of Arabidopsis roots

Arabidopsis seedling roots were stained with 30 μg/mL PI (Sigma Aldrich, USA) for 1 min and then observed. Excitation and emission wavelength were set as 535 nm and 617 nm, respectively for fluorescence microscopy (Carl Zeiss, Germany).

GENE ACCESSION NUMBERS AND SEED SOURCE

The main gene accession numbers mentioned in this article are as follows: *PARP1* (At2g31320), *PARP2* (At4g02390), *AUX1* (At2g38120), *ARF7* (At5g20730), *ARF19* (At1g19220). The seed stock numbers of the mutants used in this study are as follows: *parp1* (GK_692A05-025067), *parp2* (SALK_140400C). The mutants *parp1*, *parp2 and parp1parp2* were obtained from de Pater's lab (Jia et al. 2013). *aux1* and *arf7arf19* mutants were obtained

from Xue's lab at Institute of Plant Physiology &Ecology in Shanghai. *DR5::GUS* and *CycB1;1::GUS* reporter lines were obtained from Wang's lab at Fudan University.

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AUTHOR CONTRIBUTIONS

X.G. designed the experiments. C.L., Q.W., W.L., Z.G. and W.W. performed the experiments. H.M. provided helpful suggestions during the whole experimental process. X.G. and P.X. wrote the article.

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SUPPORTING INFORMATION

Figure S1. PARP inhibitor 6(5H)-phenanthridinone improves *Arabidopsis* lateral root development

Arabidopsis were grown on 1/2MS plates without (control) or with 2 µM

6(5H)-phenanthridinone for 17 days and then lateral root numbers were counted. Values are the mean ±*SE*, n≥15. Significance of difference was determined by Student's *t*-test. **P*<0.05.

Figure S2. The lateral root elongation is accelerated when PARP activities are inhibited

(**A**) GUS staining results of *CYCB1;1::GUS* reporter lines grown on control plate. (**B**) GUS staining results of *CYCB1;1::GUS* reporter lines grown on 3-AB plate. (**C**) The numbers of embedded, emerged and total lateral roots in plants grown on control plate and 3-AB plate, respectively from (**A**) and (**B**). LR, lateral root. (**D**) Comparison of the primary root lengths of control and 3-AB treated seedlings in (**A**) and (**B**). One-week-old seedlings were observed. 1 mM 3-AB was used for the experiments. Red arrowheads indicate the initiation sites of lateral roots. Values are shown as the mean ±*SE*, n≥15; Significance of difference was determined by Student's *t*-test. **P*<0.05; ***P*<0.01.

Figure S3. Phenotypes of Col-0 and the *aux1* mutant grown on control plate and 3-AB plate, respectively

The plants were grown on plates for 12 days. Bar=1cm. 3-AB, 1mM

Figure S4. The wild type Col-0 and *the parp1parp2* mutant respond similarly to IAA treatments

(**A**) The lateral root numbers of Col-0 and the *parp1parp2* mutant grown on control plate, 1nM, and 1 μM IAA plate respectively for 22 days. LR, lateral root. (**B**) The primary root lengths of Col-0 and the *parp1parp2* mutant grown on

control plate, 1nM, and 1 μM IAA plate respectively for 22 days. PR, primary root. Values are shown as the mean ±*SE*, n≥15; Significance of difference was determined by Student's *t*-test.

Figure S5. PARP1 interacts with PARP2 *in vivo* and *in vitro*

(**A**) Yeast two hybrid assay showing that PARP1 can interact with PARP2 in yeast. β-galactosidase filter assay was used to confirm the interaction. a, positive control; b, negative control; c, sample; d and e, controls for c. (**B**) BiFC assay showing that PARP1 interacts with PARP2 in tobacco epidermal cells. The positive control infiltrated with agrobacteria carrying 35S::YFP, and two negative controls infiltrated with that carrying 35S::PARP1-YFP^N+35S::YFP^C and 35S::YFP^N+35S::PARP2-YFP^C respectively. The imaging settings for all samples are identical.

Figure S6. The working model of PARP1 and PARP2 in root development

Table S1. Real-time RT-PCR primer list

Report S1. Two-way ANOVA analysis results of Figure 5B

Report S2. Two-way ANOVA analysis results of Figure 5C

FIGURE LEGENDS

Figure 1. 3-AB inhibits PARP activities *in vitro* and *in vivo*

(**A**) Auto-modification activities of the recombinant PARP1 and PARP2 proteins result in electrophoresis mobility shift on SDS-PAGE gel which can be inhibited by 3-AB. The red lines beside the wells indicate the up-shifted proteins. (**B**) Immuno-blot analysis using anti-PAR antibody showing that the PARP activities are inhibited by 3-AB. The pictures of the experimental result with or without 3-AB are from the same blot. (**C**) Dot blot showing that the PAR synthesis induced by zeocin in the total root protein extracts is reduced by 3-AB. (**D**) Immuno-blot analysis using anti-PARP2 antibody showing that in vivo PARP2 activity is inhibited by 3-AB. The red line indicates the up-shifted modified PARP2 protein. Total proteins were extracted from two-week-old *Arabidopsis* seedlings treated by zeocin or zeocin+3-AB for 24 h. Control plants were treated with the same amount of water. Red arrowheads indicate the positions of the target proteins. Zeocin, 200 μg/mL; 3-AB, 1mM.

Figure 2. *Arabidopsis* seedlings have more developed root systems when PARP inhibitor 3-AB is applied

(**A**, **B**) Phenotypes of three-week-old *Arabidopsis* seedlings grown on horizontally placed control plate and 3-AB plate respectively. Bar=0.5 cm. (**C**) Comparison of the lateral root numbers and leaf numbers of plants from (**A**). All emerged lateral roots including secondary lateral roots were counted. (**D**) Comparison of the fresh weights of plants from (**A**). (**E**) Temporal growth curves of the lateral roots of plants grown vertically on control and 3-AB plates.

Emerged lateral roots were counted. (**F**) Elongation rates of the lateral roots of plants grown vertically on control and 3-AB plates. Emerged lateral roots longer than 3mm were analyzed. (**G**) Total lateral root lengths of the plants grown vertically on control and 3-AB plates. Emerged lateral roots were measured.LR, lateral root. 1 mM 3-AB was used for all experiments. Experiments were repeated at least three times and similar results were obtained. Values are presented as the mean±*SE*, n≥15. Significance of difference was determined by Student's *t*-test. **P*<0.05; ***P*< 0.01.

Figure 3. The lateral root emergence is accelerated when PARP activities are inhibited

(**A**) GUS staining results of *DR5::GUS* reporter lines grown on control plate and 3-AB plate. Bar=0.2cm. The enlarged pictures show the primordia embedded in the primary root. Red arrowheads indicate the initiation sites of lateral roots. (**B**) The primary root lengths of plants grown on control plate and 3-AB plate from (**A**). PR, primary root. (**C**) The numbers of emerged, embedded and total lateral roots of plants grown on control plate and 3-AB plate from (**A**). LR, lateral root. Emerged, emerged lateral roots; Embedded, lateral roots embedded in PR. Total, emerged lateral roots plus embedded lateral roots. 10-day-old seedlings were observed. 1 mM 3-AB was used for the experiments. The experiments were done in triplicate. Values are shown as the mean±*SE*, n≥15. Significance of difference was determined by Student's *t*-test. **P*<0.05; ***P*< 0.01.

Figure 4. Inhibition of PARP activities enhance cell division while repress

endoreplication in root development

(**A**) Comparison of nuclear ploidy levels of plants grown on control plate and 3-AB plate, respectively. 2C- diploid, 4C- tetraploid, 8C- octaploid, 16C- hexadecaploid. (**B**) Expression level analysis of cell cycle phase transition marker genes. (**C**) Propidium iodide stained root meristems of plants grown on control plate and 3-AB plate, respectively. Arrowheads indicate the boundary between meristematic zone and elongation zone. Bar=100 μm. (**D**) Comparison of the root meristematic cell numbers of plants grown on control plate and 3-AB plate, respectively. In (**A**) and (**B**), one-week-old seedlings were used. The seedlings were treated by 3-AB for 24 h before material collection in (**B**). In (**C**) and (**D**), two-week-old seedlings were observed. 1 mM 3-AB was used in all assays. Values are presented as the mean±*SE*. Significance of difference was determined by Student's *t*-test. **P*<0.05; ***P*<0.01.

Figure 5. PARP inhibitor plays a role in a pathway distinct from auxin signaling.

(**A**) *Arabidopsis* treated with different conditions including: no treatment (w/o IAA and 3-AB), 1nM IAA, 1μM IAA, 1nM IAA+1mM 3-AB, 1μM IAA+1mM 3-AB. Bar=0.5 cm.(**B**) The primary root lengths of plants treated with different conditions including: no treatment (w/o IAA and 3-AB), 1nM IAA, 1μM IAA, 1nM IAA+1mM 3-AB, 1μM IAA+1mM 3-AB. (**C**) The lateral root numbers of plants treated with different conditions including: no treatment (w/o IAA and 3-AB), 1nM

IAA, 1μM IAA, 1nM IAA+1mM 3-AB, 1μM IAA+1mM 3-AB. (**D**) The lateral root numbers of Col-0, *aux1* and *arf7/arf19* mutants grown on control plate and 3-AB plate, respectively. For(**A**), (**B**) and (**C**), two-week-old seedlings were observed. For (**D**), 12-day-old seedlings were observed. Experiments were repeated in triplicate. Values are presented as the mean±*SE*, n=20. Significance of difference was determined by two-way ANOVA test for (**B**) and (**C**) (the results are shown in ReportsS1 and S2), and Student's*t*-test for (**D**). **P*<0.05; ***P*<0.01.

Figure 6. *The parp1parp2* double mutant has more developed roots than wild type plants

(**A**) The phenotype of the *parp1parp2* mutant grown on control plate and 1mM 3-AB plate respectively. Bar=1cm. (**B**) Comparison of the lateral root numbers of Col-0 and the *parp1parp2* plants grown on control plate and 1 mM 3-AB plate respectively. LR, lateral root. (**C**) Comparison of the primary root lengths of Col-0 and the *parp1parp2* plants grown on control plate and 1 mM 3-AB plate respectively. PR, primary root. 12-day-old seedlings were observed for this experiment. The experiment was repeated in triplicate. Values are presented as the mean±*SE*, n=15. Significance of difference was determined by Student's *t*-test. ***P*<0.01, **P*<0.05.

Figure 7. Expression levels of *PARP1* and *PARP2* in roots

(**A**,**B**) GUS staining results of *PARP2::GUS* transgenic line showing that *PARP2* is more abundantly expressed in the differentiation zone but less in the meristematic zone of the primary root. (**A**, **B**) are from different plants. Bar=100

μm. (**C**,**D**) GUS staining results of *PARP2::GUS* transgenic line showing that *PARP2* is less expressed in the meristematic zone but more abundantly in the elongation and differentiation zones of lateral roots. (**C**,**D**) are from different plants. Bar=100 μm. The enlarged area in (**D**) showing that the elongation zone of the lateral root just after emergence. Expression of *PARP2* can be observed in the elongation zone.(**E**) Expression levels of *PARP1* and *PARP2* in one-week old seedlings. In addition to the whole seedlings, the aerial parts of the seedlings and the root tips about 0.5 cm long were used for the examination. Two-week-old plants were used for (**A**) to (**D**). mz, meristematic zone; ez, elongation zone; dz, differentiation zone. Significance of difference was determined by Student's *t*-test. ***P*<0.01.

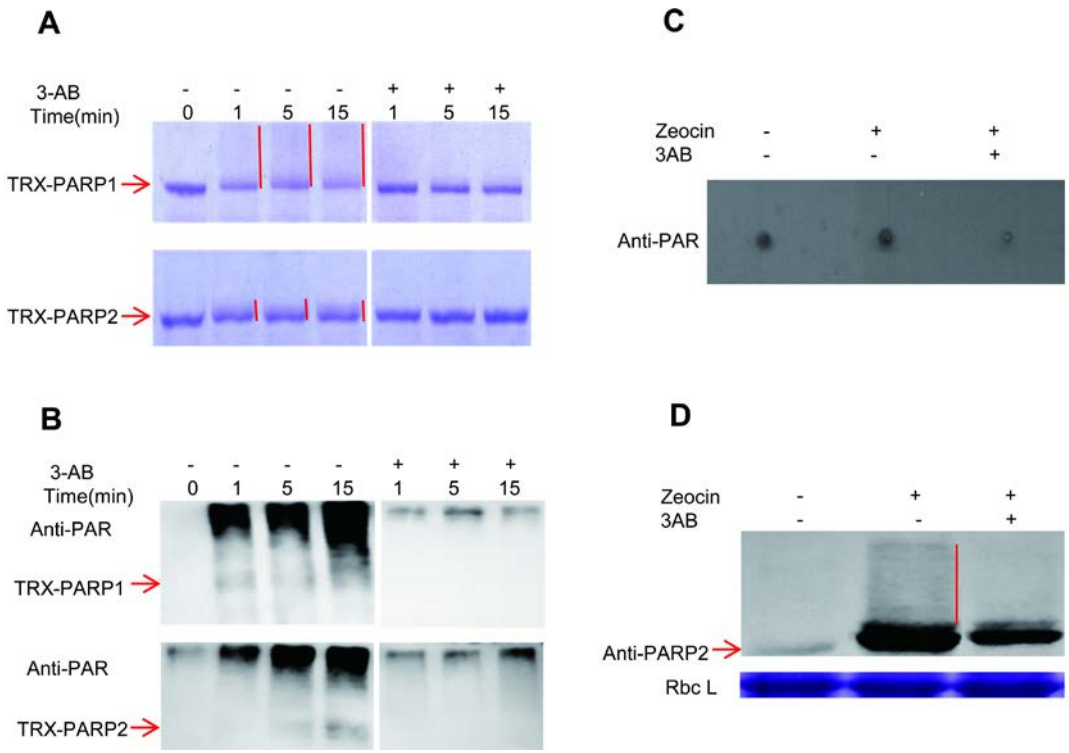

Figure 1

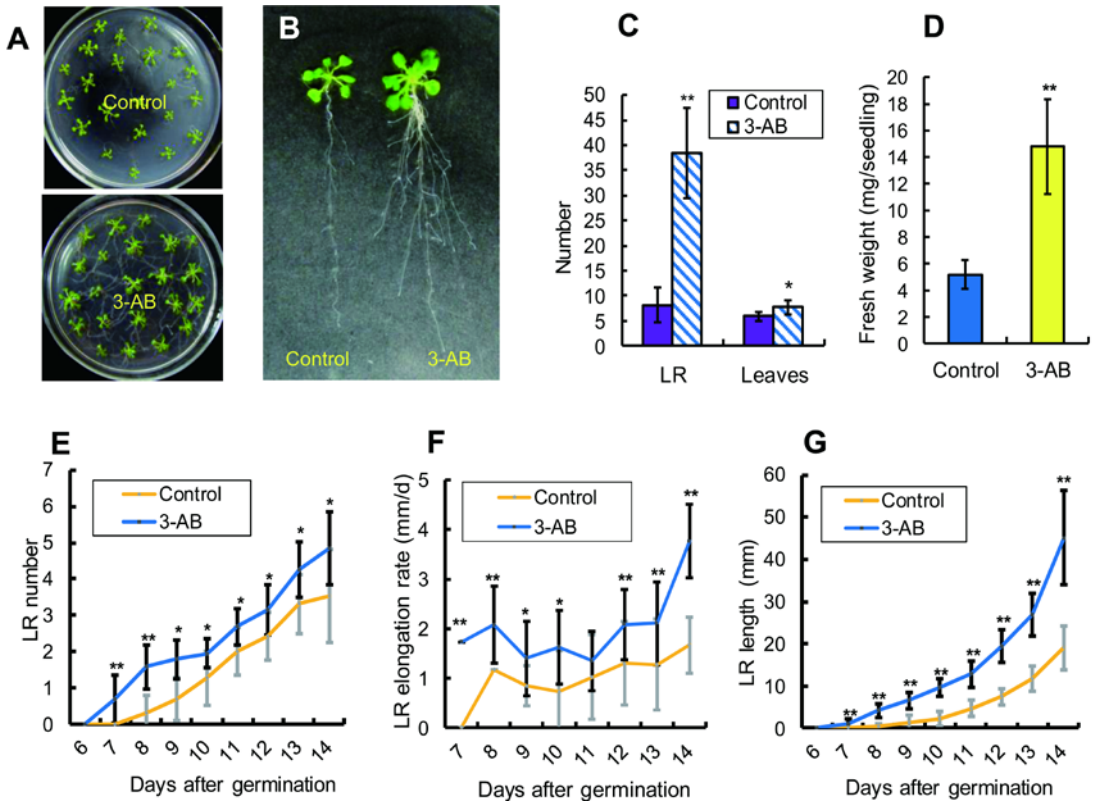

Figure 2

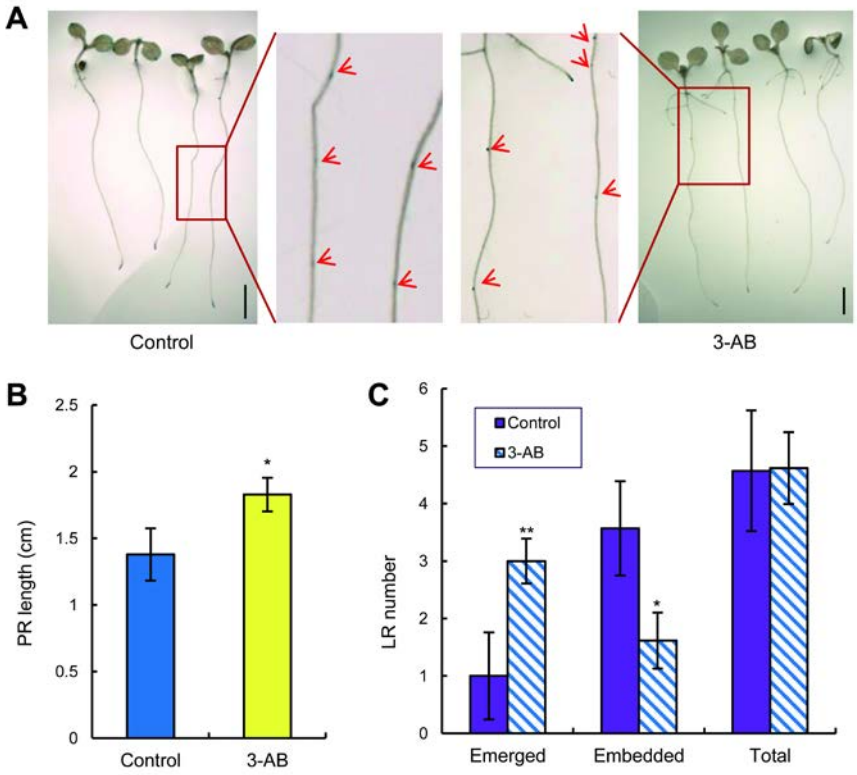

Figure 3

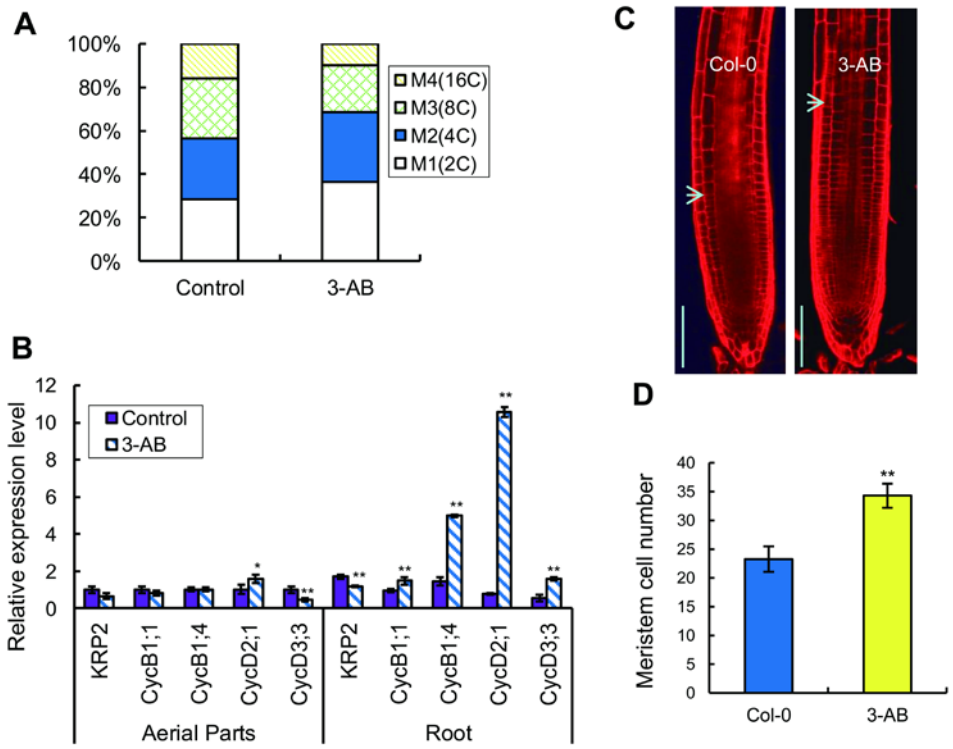

Figure 4

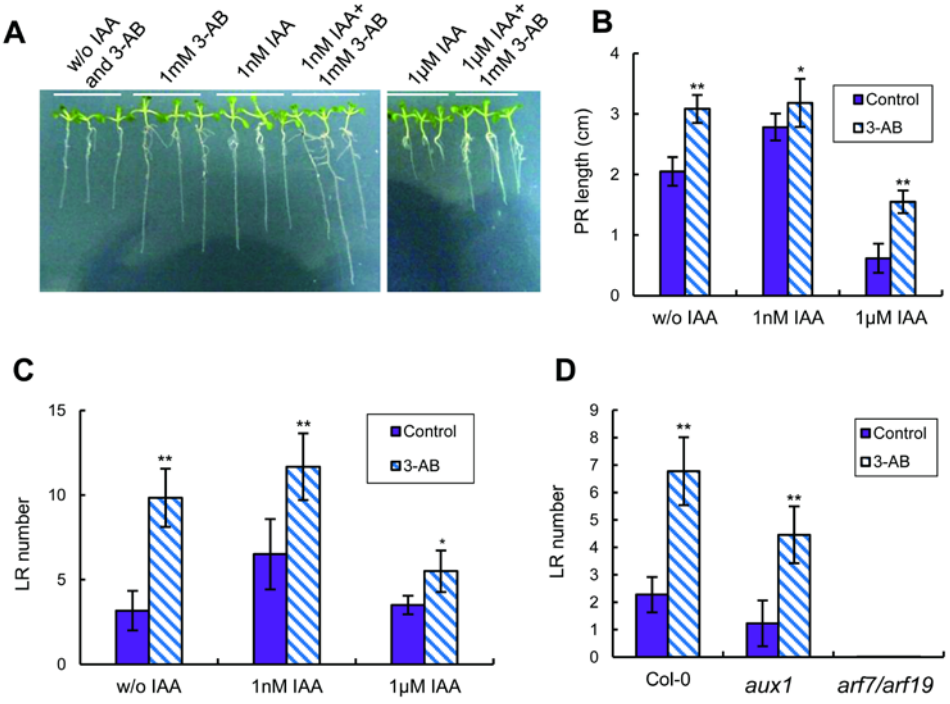

Figure 5

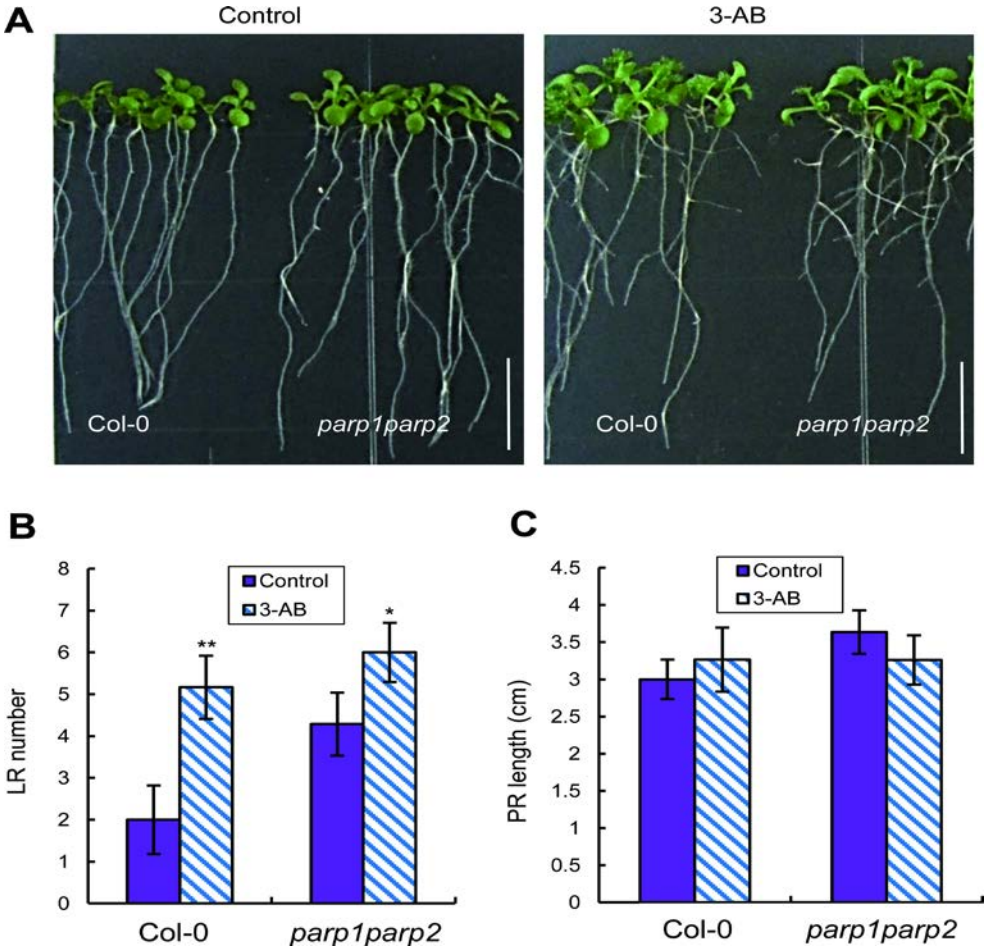

Figure 6

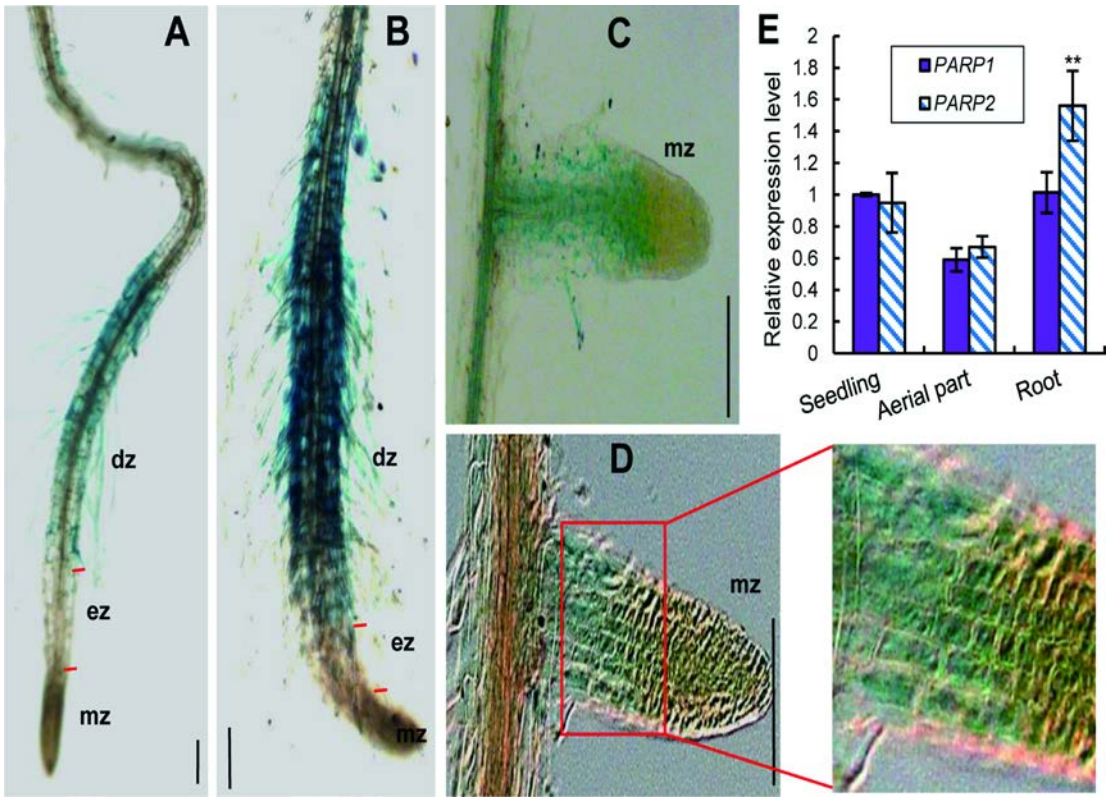

Figure 7