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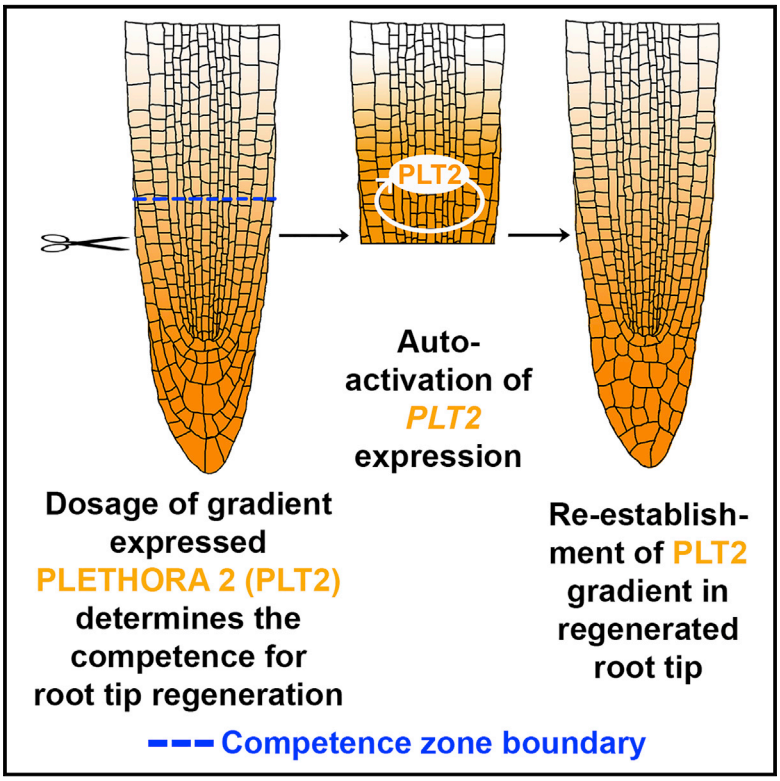
Gradient Expression of Transcription Factor Imposes a Boundary on Organ Regeneration Potential in Plants

Graphical Abstract



Highlights

- The developmental gradient of PLT2 correlates with organ regeneration competence
- PLTs are essential for primary as well as lateral root tip regeneration
- PLT2 confers regeneration potential to differentiating cells via auto-activation
- The regeneration potential of root meristem can be decoupled from its size

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In Brief

Durgaprasad et al. show that dosage of a gradient-expressed transcription factor orchestrates the regeneration competence in developing root tip. Interestingly, the regeneration potential of root meristem can be separated from its size.

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Gradient Expression of Transcription Factor Imposes a Boundary on Organ Regeneration Potential in Plants

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SUMMARY

A wide variety of multicellular organisms across the kingdoms display remarkable ability to restore their tissues or organs when they suffer damage. However, the ability to repair damage is not uniformly distributed throughout body parts. Here, we unravel the elusive mechanistic basis of boundaries on organ regeneration potential using root tip resection as a model and show that the dosage of gradient-expressed PLT2 transcription factor is the underlying cause. While transient downregulation of PLT2 in distinct set of *plt* mutant backgrounds renders meristematic cells incapable of regeneration, forced expression of PLT2 acts through auto-activation to confer regeneration potential to the cells undergoing differentiation. Surprisingly, sustained exposure to nuclear PLT2, beyond a threshold, leads to reduction of regeneration potential despite giving rise to longer meristem. Our studies reveal dosage-dependent role of gradient-expressed PLT2 in root tip regeneration and uncouple the size of an organ from its regeneration potential.

INTRODUCTION

Plants and animals display a remarkable potential to regenerate damaged organs during their life cycles (Sánchez Alvarado and Tsonis, 2006; Birnbaum and Sánchez Alvarado, 2008; Tanaka and Reddien, 2011). Plants can recreate their entire body in response to external inductive cues (Pulianmackal et al., 2014). Both developmental regulators and wound-responsive factors are involved in whole-plant regeneration from assemblies of cells (Ikeuchi et al., 2016). *In vitro* regeneration studies indicate a two-step process for whole-plant regeneration, in which acquisition of regeneration competence can be separated from the execution of a new tissue differentiation program (Atta et al., 2009; Che et al., 2007; Kareem et al., 2015). When plant organs are severed, they are able to regenerate missing tissues (van den Berg et al., 1995; Feldman, 1976; Reinhardt et al., 2003; Schiavone and Racusen, 1991). However, the factors that define competence for the partial regeneration of damaged organs are poorly understood.

Similar to teleost fish that exhibit differential regeneration capability along the proximo-distal axis of the caudal fin (Morgan, 1902), roots of the model plant *Arabidopsis* exhibit differences in regeneration efficiency along the root-shoot axis. Damage-induced regeneration competence is confined mostly to the rootward regions of the root meristem, although all meristematic cells are capable of dividing (Sena et al., 2009). The decline in the frequency of regeneration toward shootward end of the root meristem is associated with massive changes in gene expression (Efroni et al., 2016), but key factors that determine the competence for regeneration after mechanical damage have not yet been identified.

The existence of differential regeneration potential among meristematic cells suggests that factors with graded expression may underlie regeneration capacity. PLETHORA proteins are expressed in root tips and are plausible candidates for the regulation of competence (Galinha et al., 2007). Four of the PLTs (PLT1, PLT2, PLT3, and PLT4) play an important role in root stem cell maintenance and exhibit functional redundancy (Galinha et al., 2007). Like most other PLTs, PLT2 protein is expressed in the shape of a gradient along the root meristem (Galinha et al., 2007). PLT2 regulates root meristem maintenance, and its concentration is critical for successive fate transitions of meristematic cells from stem cell to transit amplifying cell and differentiating cell (Mähönen et al., 2014). Moreover, the regeneration of root tips following laser ablation of quiescent center (QC) is impaired in *plt1plt2* mutants (Xu et al., 2006). Although PLT2 has been studied in the context of root tip regeneration (Sena et al., 2009; Xu et al., 2006), its potential role in regeneration competence is hitherto unexplored. Here, we use root tip resection as a model and show that the developmental

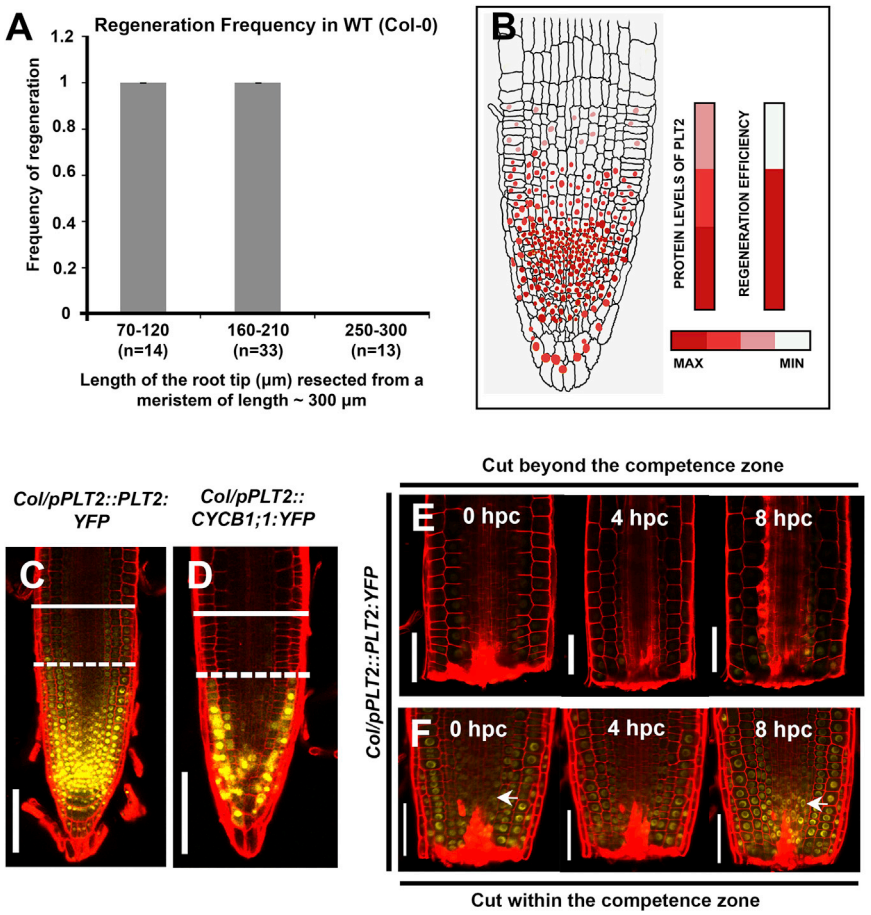

Figure 1. PLT2 Protein Levels Correlate with Regeneration Competence

(A) Regeneration frequency in WT (Col-0) root with a meristem of length ~300 μm; error bars represent SEM. For all the measurements presented in the graph, only WT (Col-0) and not any reporter line was used. Refer to Method Details for the measurement of the size of resected root tip and meristem length.

(B) Schematic representation of relation between protein levels of PLT2 and regeneration efficiency. The PLT2 protein gradient shown is drawn on the basis of actual data.

(C and D) Confocal image of *Col/pPLT2::PLT2:YFP* (C), expressing pPLT2::PLT2:YFP, showing overlap between high PLT2 protein levels and the competence zone. Confocal image of *Col/pPLT2::CYCB1;1:YFP* (D), expressing pPLT2::CYCB1;1:YFP, showing overlap between actively dividing cells (marked with CYCB1;1:YFP) in PLT2 transcriptional domain and the competence zone. Dashed lines mark the boundary of competence zone, and solid lines mark the boundary of root meristem. Red cell outline is due to propidium iodide staining. Scale bars: 100 μm.

(E and F) Confocal images of single roots of *Col/pPLT2::PLT2:YFP*, expressing pPLT2::PLT2:YFP, during first 8 h post-resection beyond the competence zone (E) and within the competence zone (F). Arrow marks the upregulation of pPLT2::PLT2:YFP at 8 hpc in (F). In (E), where root is resected beyond the competence zone, only a few cells show increase in pPLT2::PLT2:YFP expression at 8 hpc, whereas in (F), where root is resected within the competence zone, many cells show significant increase in pPLT2::PLT2:YFP expression. Thus, the level of pPLT2::PLT2:YFP is increased and its domain is expanded in the competence zone compared with beyond the competence zone. Scale bars: 50 μm.

gradient of PLT2 correlates with the competence for root tip regeneration. We reveal that decline of PLT2 toward the shootward end of meristem is causal for the drop in regeneration capability at this region. Moreover, we show the necessity of PLT2 in the regeneration of primary root tip as well as lateral root tip by its transient downregulation in growing roots of multiple *plt* mutant backgrounds. Finally, PLT2 is sufficient to trigger root tip regeneration from regeneration-incompetent differentiating root cells.

RESULTS

Position of Cells within the PLT2 Gradient Correlates with Regeneration Competence

PLT proteins constitute a plant-specific family of AP2-domain transcription factors, and most of its members have their expression domains within the competence zone. In line with previously published results (Sena et al., 2009), only roots resected at the rootward end of meristems in wild-type (WT), where PLT2::YFP levels were high, regenerated. Roots resected close to the boundary of the meristem, where PLT2 levels were low, did not regenerate (Figures 1A, 2A, and 2B). Resections that generated sharp cuts produced little damage at the cut ends, while resections that generated blunt cuts produced more damage at the cut ends. Since resections that generated blunt cuts lead to no regeneration of the root tips due to more damage at the cut end, only resections that produced sharp cuts were considered in all our analysis, to be consistent with the technique and to avoid any variation due the mechanical injury while comparing the data collected from the competence zone and beyond competence zone (Figures 2C and 2D). We quantified the levels of PLT2::YFP along the root-shoot axis of the meristem and observed a striking correlation between PLT2 levels and regeneration potential of the root (Figures 1B, 2E, and 2F). This led us to define the zone of regeneration competence. In line with this correlation, the transcriptional domain of *PLT2* (examined using a transgenic line, *Col/pPLT2::CYCB1;1:YFP*; Mähönen et al., 2014), previously shown to overlap only with the high-expression domain of PLT2::YFP (Mähönen et al., 2014), coincided with the competence zone for regeneration (Figures 1C and 1D).

Interestingly, we noticed significant upregulation of PLT2 as early as 8 h post-cut (hpc) in the roots resected within the competence zone (Figures 1E, 1F, and 2G–2I). This local upregulation at the new rootward end of the resected root was found prior to any detectable morphological change. In addition, the PLT2 gradient, which was perturbed upon resection within the competence zone, showed progressive re-establishment during regeneration (Figure 2J). We did not observe significant

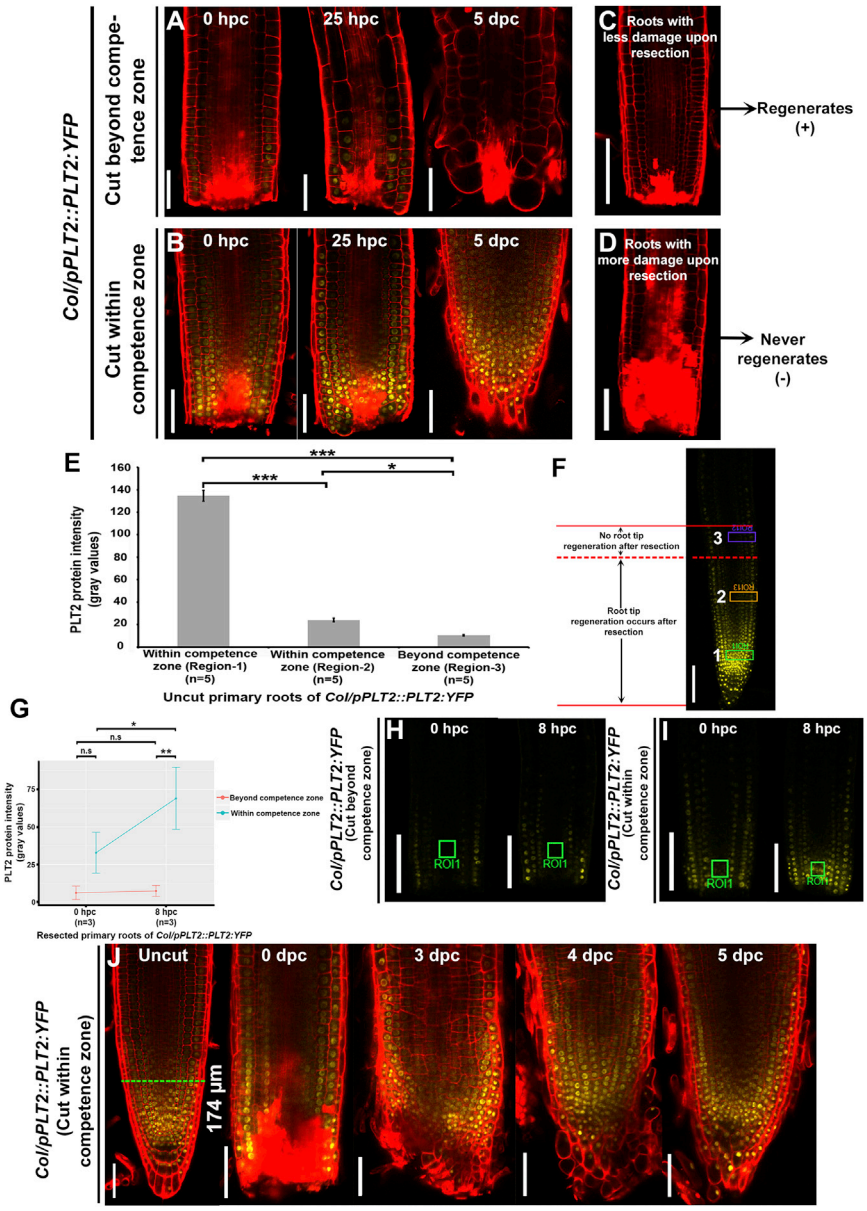

Figure 2. PLT2 Protein Levels Are High within the Competence Zone, and PLT2 Gradient Is Re-established When Resected within Competence Zone

(A and B) Confocal images of non-regenerating root (A) and regenerating root (B) of *Col/pPLT2::PLT2:YFP*, expressing pPLT2::PLT2:YFP, when resected beyond the competence zone and within the competence zone, respectively. Long-term consequences on pPLT2::PLT2:YFP expression and root regeneration, when resected within and beyond the competence zone, are shown in this figure. Scale bar: 50 μm.

(C and D) Confocal images of resected roots with less damage (C) and more damage (D); damage is caused by resection. In our experiments, only the resected roots with sharp cuts, as shown in (C), regenerated. Because of technical failure, the resected roots with blunt cuts contained more damage, as shown in (D), and they never regenerated. Therefore, to avoid any variations, we considered only resected roots with sharp cuts that displayed regeneration for all our analysis. We completely discarded all the resected roots with blunt cuts, containing more damage, which were caused by technical failure. Scale bar: 50 μm.

(E) Significant difference in PLT2 protein intensity among region 1, region 2 (present within the competence zone), and region 3 (present beyond the competence zone) in the primary roots of *Col/pPLT2::PLT2:YFP* (ANOVA: degrees of freedom [df] = 2, F = 495.8, p = 2.92 × 10^{-12}). Significant difference in PLT2 protein intensity between region 1 and region 2, present within the competence zone in the primary roots of *Col/pPLT2::PLT2:YFP* (Tukey's honestly significant difference [HSD] test: $p < 0.001$). Significant difference in PLT2 protein intensity between region 1, present within the competence zone, and region 3, present beyond the competence zone, in the primary roots of *Col/pPLT2::PLT2:YFP* (Tukey's HSD test: $p < 0.001$). Significant difference in PLT2 protein intensity between region 2, present within the competence zone, and region 3, present beyond the competence zone, in the primary roots of *Col/pPLT2::PLT2:YFP* (Tukey's HSD test: p = 0.0216925). Asterisks illustrate the p value: *$p < 0.05$ and ***$p < 0.001$. Error bars represent SEM.

(F) Confocal image of the root of *Col/pPLT2::PLT2:YFP* expressing pPLT2::PLT2:YFP. This is a representative image of the roots considered for quantification of PLT2 protein intensity in (E). Boxed regions were considered for quantification of PLT2 protein intensity in five independent roots of *Col/pPLT2::PLT2:YFP*. Region 1 and region 2 lie within the competence zone, while region 3 lies beyond the competence zone. The dimensions of all three regions were maintained the same, in all roots considered for quantification in (E). These regions contain lateral root cap cells, epidermal cells, cortical cells, endodermal cells, pericycle cells, and vascular cells. Dashed line marks the boundary of competence zone, and solid line marks the boundary of root meristem. Scale bar: 100 μm.

(G) Significant difference in PLT2 protein intensity among the primary roots of *Col/pPLT2::PLT2:YFP*, at 0 and 8 hpc, when resected within the competence zone and beyond the competence zone (ANOVA: df = 3, F = 16.11, p = 0.000942). Significant difference in PLT2 protein intensity between 0 and 8 hpc, in the primary roots of *Col/pPLT2::PLT2:YFP*, resected within the competence zone (Tukey's HSD test: p = 0.0335507). No significant difference in PLT2 protein intensity between 0 and 8 hpc, in the primary roots of *Col/pPLT2::PLT2:YFP*, resected beyond the competence zone (Tukey's HSD test: p = 0.9993629). No significant difference in PLT2 protein intensity at 0 hpc between the primary roots of *Col/pPLT2::PLT2:YFP*, resected within the competence zone and beyond the competence zone (Tukey's HSD test: p = 0.1223324). Significant difference in PLT2 protein intensity at 8 hpc between the primary roots of *Col/pPLT2::PLT2:YFP*, resected within the competence zone and beyond the competence zone (Tukey's HSD test: p = 0.0015584). The data are paired, and the same plants of *Col/pPLT2::PLT2:YFP* were taken at 0 and 8 hpc time points, when resected within the competence zone as well as beyond the competence zone. Asterisks illustrate the p value: *$p < 0.05$ and **$p < 0.01$. Error bars represent SD.

(H and I) Confocal images of the roots of *Col/pPLT2::PLT2:YFP* expressing pPLT2::PLT2:YFP at 0 and 8 hpc when resected beyond the competence zone (H) and within the competence zone (I). These are representative images of the roots considered for quantification of PLT2 protein intensity in (G). Boxed region was considered for quantification of PLT2 protein intensity in three independent roots of *Col/pPLT2::PLT2:YFP*, when resected beyond the competence zone

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upregulation of PLT2 at 8 hpc in the roots resected beyond the competence zone (Figures 1E, 2G, and 2H). Overall, our results reveal a correlation between tissues with high levels of PLT2 and competence for regeneration and suggest that the dosage of gradient-expressed PLT2 determines the competence for regeneration.

PLTs Are Essential for Organ Regeneration in Primary and Lateral Roots

Because *plt1plt2* double mutants still regenerate, albeit with lower frequency compared with WT roots (Sena et al., 2009), we sought to overcome functional redundancy and test the specific role of PLT2 in regeneration of primary root tips. To this end, we used a *PLT2* translational fusion construct tagged to *YFP* and an inducible RNAi construct of *PLT2* in *plt1plt2+/−plt3plt4* mutant background (*plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi*; *plti* from here on) (Mähönen et al., 2014). In this line, RNAi silencing of *PLT2* is driven by an estradiol-inducible ubiquitous promoter (Siligato et al., 2016). Prior to induction, the morphology, meristem length, competence zone length, and regeneration efficiency of *plti* primary roots was similar to that of WT primary roots (Figures S1A and S1B). After 47 h induction, the PLT2::YFP in *plti* primary roots reached a lower level than observed beyond the competence zone of uninduced roots, while the meristem length was similar to that of uninduced roots (Figures 3A, 3B, and S1C–S1E). The low PLT2::YFP levels at this time point serves as a readout of transient downregulation of endogenous *PLT2* expression. Therefore, at 47 h induction, we examined the regeneration efficiency upon resection within the competence zone in *plti* primary roots, where PLT2 was transiently downregulated and other redundant PLTs were absent. Interestingly, these roots did not regenerate, while uninduced primary roots resected within the competence zone did regenerate (Figures 3C and 3D). This demonstrated the necessity of the PLT2 protein for the regeneration of the primary root tip in the absence of three redundant sister proteins.

We next investigated whether the requirement for PLT2 also was critical in another developmental context, the lateral root (LR). We used the *plt3plt5plt7* mutant background in which only primary roots develop. Though LR primordia (LRP) originate in this triple mutant, they do not grow out into LRs. Other PLTs (PLT1, PLT2, and PLT4) are not expressed in the LRP of the triple mutant, and reintroduction of any PLT clade member in triple-mutant LRP completely restores LR outgrowth (Du and Scheres, 2017). We exploited this feature to make a PLT2-inducible system for LRs by introducing *PLT2* fused to the glucocorticoid receptor (GR) into the LRP of triple mutant by using the *PLT3* promoter, which is active in triple-mutant LRP (*plt3,5,7/pPLT3::PLT2:GR*) (Kareem et al., 2015). In accordance with the previous study (Du and Scheres, 2017), we rescued LR outgrowth upon dexamethasone (Dex) induction with meristem length and morphology similar to WT LRs (Figures 3E and 5E–5G). Upon transfer of the induced plants with LRs to non-inductive plates, the LR meristem was gradually consumed, which culminated in the complete differentiation of the root tip (Figures 3F and 3G). To select the right timing of PLT2 downregulation by Dex, we transformed triple mutant with transcriptional fusion construct of *SCARECROW* (*SCR*) (*plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP*). SCR is a transcription factor and is expressed in QC, initial cells for the ground tissue, and endodermal cells in post-embryonic root (Wysocka-Diller et al., 2000), and its expression in LRP is controlled by *PLT* genes (Du and Scheres, 2017). Consistently, upon Dex induction of PLT2, *SCR* promoter was activated in LRs (Figure 3H). At early phases after re-transfer of plants to non-inductive plates, *SCR* promoter activity first declined in QC cells and ground tissue initials and only at later phases from endodermal cells with concomitant reduction in the meristem length (Figures 3I and 3J).

Next we used SCR expression to select LRs of triple mutant (*plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP*), with a meristem not yet differentiated. Since *SCR* promoter activity in the LRs of triple mutant is turned on only after Dex induction of PLT2, loss of *SCR* promoter activity in the QC cells and initials acts as a readout of transient downregulation of PLT2. Upon resections within the competence zone, these LRs did not regenerate but showed unorganized cellular proliferation at the resection site (Figure 3K). Our study demonstrates the necessity of *PLT2* in LR tip regeneration, consistent with a previous finding of reduced primary root tip regeneration frequency in *plt1plt2* and *scr* mutant (Sena et al., 2009). On the contrary, the LRs under continuous induction regenerated when resected within the competence zone (Figure 3L). We concluded that *PLT2* gene expression is also critical for regeneration of LR tips when its sister genes are not expressed.

PLT2 Is Sufficient to Confer Regeneration Potential beyond the Competence Zone

plt1plt2 mutants have shorter roots and reduced root meristem size early after germination (Figure 4A) and undergo complete differentiation afterward (Aida et al., 2004). At 4 days post-germination (dpg), the competence zone of the mutant root was reduced in size compared with that of WT root. We asked whether reintroduction of PLT2 at root tip of the mutant could restore the competence for root tip regeneration. For this purpose, *PLT2* was driven by the stem cell niche-restricted *CYP79B3* promoter in the *plt1plt2* mutant (*plt1plt2/pCYP79B3::PLT2:YFP*) (Mähönen et al., 2014). *PLT2* is transcribed in the stem cell niche (SCN) of *plt1plt2/pCYP79B3::PLT2:YFP*, but the protein gradient expanded shootwards because of growth dilution and cell-to-cell movement (Figure 4A). At 4 dpg, the root meristem of *plt1plt2/pCYP79B3::PLT2:YFP* was similar to that of *plt1plt2*, in terms of morphology and size

as well as within the competence zone. The dimensions of this region were maintained the same in all roots considered for quantification in (G). This region contains pericycle cells and vascular cells. Scale bars: 100 μm.

(J) Confocal images of regenerating root of *Col/pPLT2::PLT2:YFP*, expressing pPLT2::PLT2:YFP, when resected within the competence zone. Progressive re-establishment of PLT2 gradient, when resected within the competence zone, is shown in this figure. Dashed line in the uncut image marks the cut site, and the distance of cut is indicated. Scale bars: 50 μm.

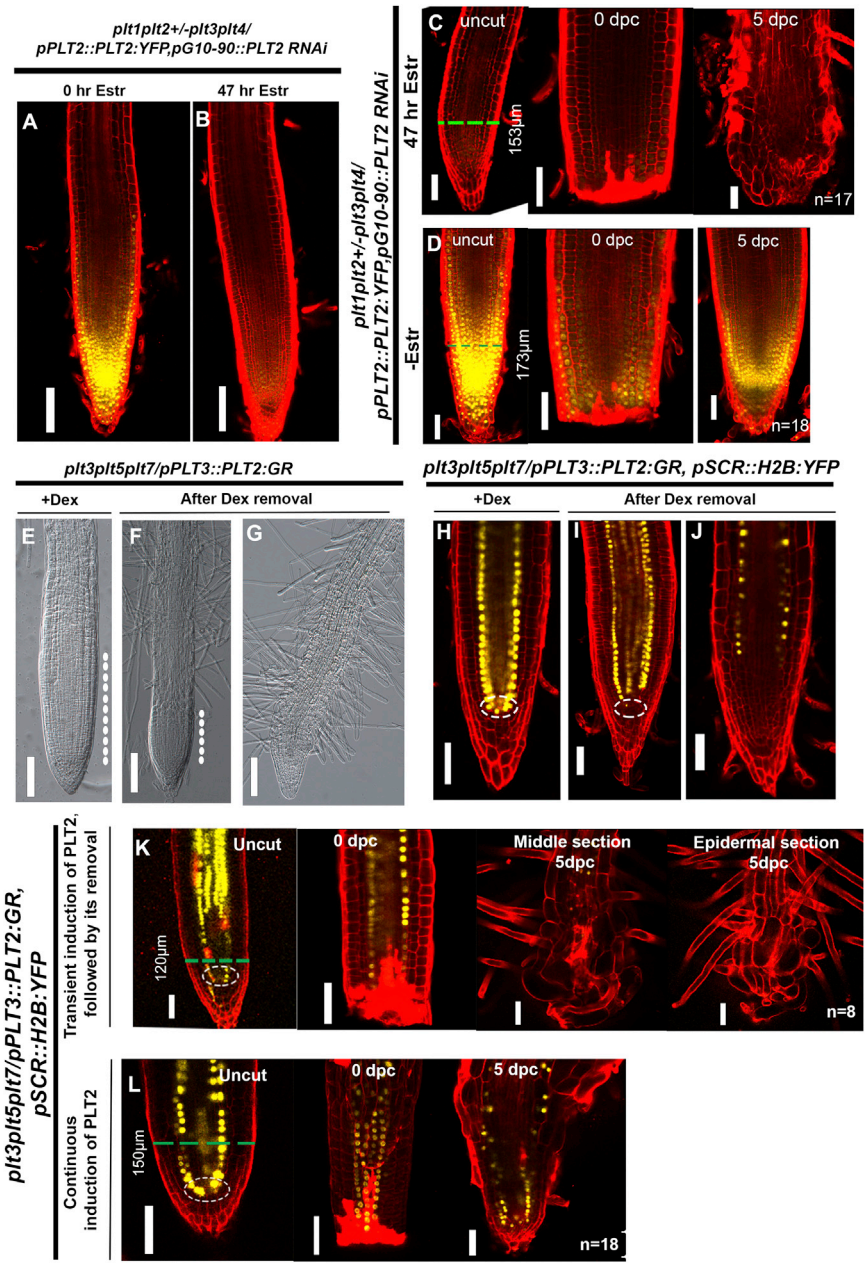

Figure 3. PLTs Are Necessary for Organ Regeneration

(A and B) Confocal images of a single root of *plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi* expressing pPLT2::PLT2:YFP, before induction (A) and after 47 h induction (B). Scale bars: 100 μm.

(C and D) Confocal images of non-regenerating root (C) and regenerating root (D) of *plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi* expressing pPLT2::PLT2:YFP, subjected to 47 h induction (C) and no induction (D), respectively. In (C), at 5 days post-cut (dpc), some unorganized cell proliferation can be observed at the cut end, but there is no repatterning, and the root tip fails to restore the missing part. Dashed lines in the uncut images mark the cut site, and the distance of cut is indicated. Scale bars: 50 μm.

(E–J) Differential interference contrast images (E–G) and pSCR::H2B:YFP expression portraying confocal images (H–J) of LRs of *plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP*. The images were taken after induction (E and H) and at consecutive stages after removal of induction (F, G, I, and J). Dotted lines drawn to the right of the images in (E) and (F) mark the length of the meristem. Ellipses in (H) and (I) mark the location of QC cells. Scale bars: 100 μm (E–G) and 50 μm (H–J).

(K and L) Confocal images of non-regenerating root (K) and regenerating root (L) of *plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP* expressing pSCR::H2B:YFP, subjected to transient induction (K) and continuous induction (L), respectively. In (K), at 5 dpc, some unorganized cell proliferation can be observed at the cut end, but there is no repatterning, and the root tip fails to restore the missing part. Dashed lines in the uncut images mark the cut site, and the distance of cut is indicated. Ellipses in the uncut images mark the location of QC cells. Scale bars: 50 μm.

See also Figure S1.

(Figure 4A), but the competence zone expanded shootward, compared with that of *plt1plt2* (Figures 4B–4D). In conclusion, regeneration competence in *plt1plt2/pCYP79B3::PLT2:YFP* can be regained by PLT2 re-expression at a stage when root meristem size and other morphological features of the root tip are still unaltered.

We next asked whether PLT2 can shift shootward, the zone of regeneration. We deployed the steroid-inducible overexpression system of *PLT2* in WT background (*Col/35S::PLT2:GR*) (Galinha et al., 2007) to deliver high levels of PLT2 beyond the competence zone. After minimally 90 min induction, regeneration ensued from root cells rootward to the elongation zone, and maximum regeneration competence occurred after 2 h induction (Figure 4K). At this stage, regeneration was triggered even from elongating cells, which was never observed in the WT control (Figures 4E and 4F). At 2 h induction, the morphology of the root used for the severing experiments had unaltered meristem size and cell division state (as indicated by the expression status of *CYCB1;1::GFP*) compared to WT control (Figures 4G–4J).

In WT roots, prior to resection as well as during early hours post-resection, many *CYCB1;1::GFP*-expressing cells were present in the competence zone, while none or very few *CYCB1;1::GFP*-expressing cells were noticed beyond the competence zone (Figures S2A–S2C). This expression pattern is unaltered in *Col/35S::PLT2:GR* uncut roots after 2 h Dex induction (Figures 4I and 4J). At 6 hpc, *CYCB1;1::GFP*-expressing cells appear at the cut site in *Col/35S::PLT2:GR*, indicating the onset of cell divisions (Figure S2E), but not in WT control

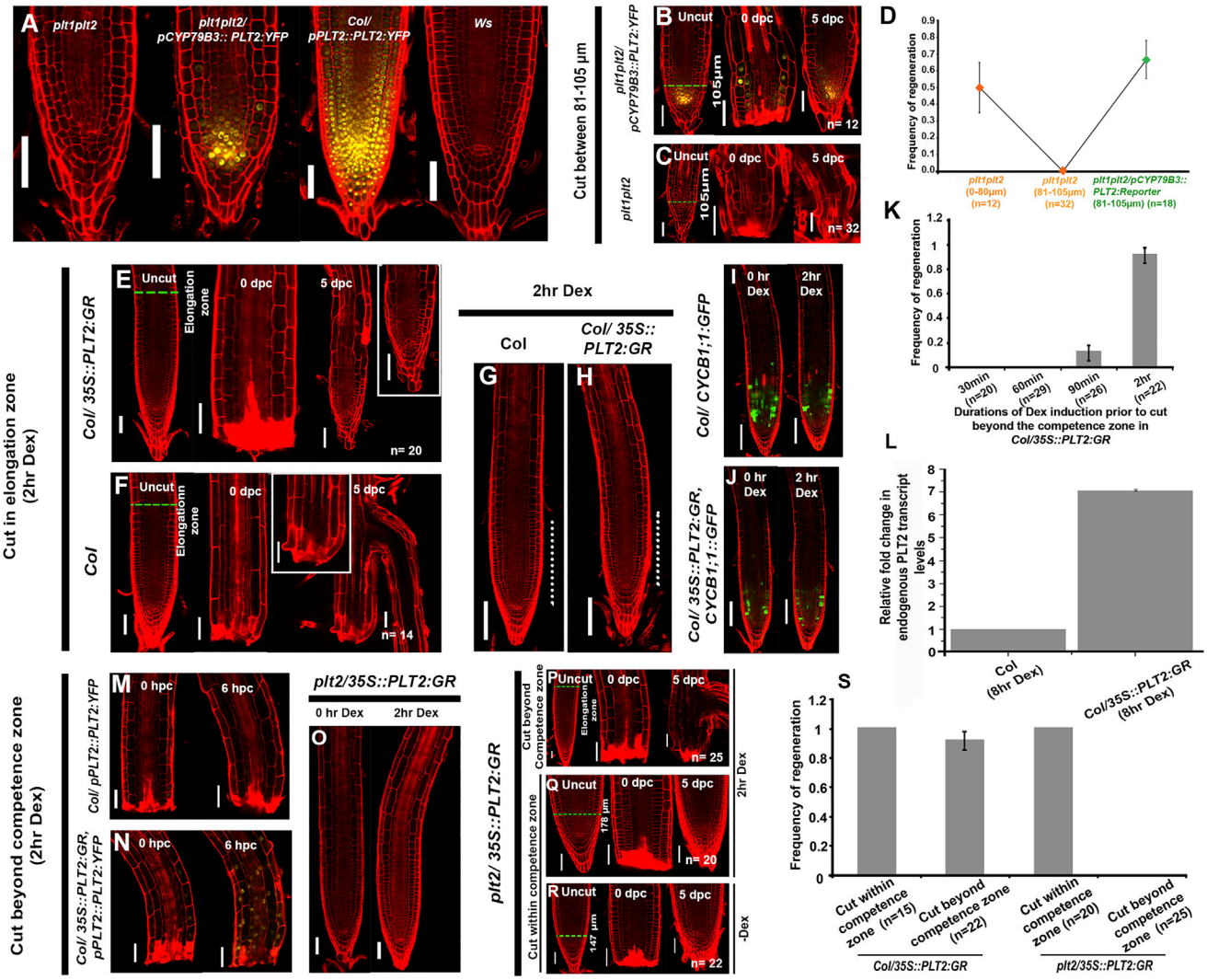

Figure 4. PLT2 Is Sufficient for Organ Regeneration, and Induction of Endogenous PLT2 Is Key to Competence Zone Expansion

(A) Confocal images of roots of *plt1plt2, plt1plt2/pCYP79B3::PLT2:YFP* expressing pCYP79B3::PLT2:YFP, *Col/pPLT2::PLT2:YFP* expressing pPLT2::PLT2:YFP and Ws. Scale bars: 50 μm.

(B and C) Confocal images of regenerating root of *plt1plt2/pCYP79B3::PLT2:YFP* expressing pCYP79B3::PLT2:YFP (B) and non-regenerating root of *plt1plt2* (C), resected between 81 and 105 μm. Dashed lines in the uncut images mark the cut site, and the distance of cut is indicated. Scale bars: 50 μm.

(D) Regeneration frequency in *plt1plt2*, when resected between 0 and 80 μm, and in *plt1plt2* as well as *plt1plt2/pCYP79B3::PLT2:Reporter (plt1plt2/pCYP79B3::PLT2:YFP or plt1plt2/pCYP79B3::PLT2:CFP)*, when resected between 81 and 105 μm; error bars represent SEM.

(E and F) Confocal images of regenerating root of *Col/35S::PLT2:GR* (E) and non-regenerating root of Col (F), resected in elongation zone. Prior to resection, the roots were subjected to Dex induction for 2 h. After resection, the roots were transferred on to a Dex-free medium. Dashed lines in the uncut images mark the elongation zone, where resection was done. Insets in (E) and (F) contain the magnified portion of the root tip at 5 dpc. Scale bars: 50 μm.

(G and H) Confocal images of roots of Col (G) and *Col/35S::PLT2:GR* (H) after 2 h induction. Dotted lines drawn to the right of the image mark the length of meristem. Scale bars: 100 μm.

(I and J) Confocal images of single root of *Col/CYCB1;1::GFP* (I) and *Col/35S::PLT2:GR*, *CYCB1;1::GFP* (J), expressing CYCB1;1::GFP, before and after 2 h Dex induction. The expression of CYCB1;1::GFP varies among roots in both *Col/CYCB1;1::GFP* and *Col/35S::PLT2:GR*, *CYCB1;1::GFP* before induction. Scale bars: 100 μm.

(K) Regeneration frequency in *Col/35S::PLT2:GR*, when resected beyond the competence zone after different durations of Dex induction; error bars represent SEM.

(L) Upregulation of endogenous PLT2 transcripts in *Col/35S::PLT2:GR*, upon 8 h Dex induction, as measured by qRT-PCR. Expression levels were normalized to *ACTIN2*. Error bar represents SEM from two independent biological replicates.

(M and N) Confocal images of single root of *Col/pPLT2::PLT2:YFP* (M) and *Col/35S::PLT2:GR, pPLT2::PLT2:YFP* (N), expressing pPLT2::PLT2:YFP, during first 6 h post-resection beyond the competence zone. Prior to resection, the roots were subjected to Dex induction for 2 h. After resection, the roots were transferred onto Dex-free medium. Scale bars: 50 μm.

(O) Confocal images of single root of *plt2/35S::PLT2:GR*, before and after 2 h Dex induction. Scale bars: 50 μm.

(legend continued on next page)

(Figure S2D). In conclusion, induction of high levels of PLT2 confers regeneration potential to differentiating root cells beyond the competence zone in which cell cycle had ceased.

Auxin has been implicated in root tip regeneration (Sena et al., 2009). We therefore examined the auxin response within and beyond the competence zone. During early hours post-resection, the auxin response marker DR5::VENUS showed a similar pattern of expression in the roots resected within competence zone as well as in the roots resected beyond the competence zone (Figures S3A–S3C). In both the cases, there was selective accumulation of DR5::VENUS signal at the cut site at 8 hpc, indicating upregulation of auxin response (Figures S3B and S3C). Since we found the same auxin response in regenerating roots as well as non-regenerating roots, we exploited another auxin sensor, DII::VENUS in *R2D2* line (Figures S3D–S3F), which is much more sensitive to fluctuations in auxin levels compared with DR5::VENUS. Intensity of DII::VENUS is inversely related to auxin levels (Brunoud et al., 2012; Liao et al., 2015; Di Mambro et al., 2017). The intensity of DII::VENUS marker dropped significantly at an earlier time point (4 hpc) in the roots resected beyond the competence zone (Figure S3E), while such a significant drop was observed only at a later time point (8 hpc) in the roots resected within the competence zone (Figure S3F). The abundance of auxin levels in the WT roots resected beyond the competence zone suggests that it is not the auxin levels but rather high levels of PLT2 that are the limiting factor for regeneration beyond the competence zone.

Induction of Endogenous PLT2 Expression Is Key to Competence Zone Expansion

So far our data suggest that maintenance of high concentrations of PLT2 is essential as well as sufficient for conferring regeneration potential to root tip. There are examples in which autoregulation of important developmental regulatory proteins maintain their required constant levels for extended developmental periods (Crews and Pearson, 2009). We therefore examined whether endogenous *PLT2* is activated upon PLT2 overexpression and whether this activation is required for maintenance of constant optimal levels of PLT2 in response to injury. We examined endogenous *PLT2* levels upon PLT2 overexpression and detected 7-fold upregulation of endogenous *PLT2* transcripts in the root tip samples of *Col/35S::PLT2:GR* subjected to 8 h induction (Figure 4L). Consistent with upregulated transcript levels, an introgressed *PLT2::YFP* transgene revealed upregulation of PLT2::YFP in 2 h Dex-induced *Col/35S::PLT2:GR* seedling in response to root tip resection. The upregulation of PLT2::YFP became more pronounced at the cut site at 6 h post-resection beyond the competence zone (Figures 4M, 4N, and S4A–S4D). The upregulation of PLT2::YFP was maintained even after removal of Dex. This indicates that the roots are competent to regenerate when resected beyond the competence zone only when upregulated PLT2::YFP is maintained at the cut site.

To explore the functional significance of endogenous PLT2 upregulation for the expansion of the competence zone, we first examined regeneration efficiency in the *plt2* mutant background. Although *plt2* mutants show a slight reduction in number of meristematic cells and increase in number of columella cells (Aida et al., 2004), regeneration efficiency of *plt2* mutant was similar to that of WT when root was resected within the competence zone. When introgressed into *35S::PLT2:GR*, root morphology remained unchanged after 2 h induction (Figure 4O). The regeneration efficiency when resected within the competence zone, where other *PLT* genes are expressed and can substitute the function of *PLT2*, also remained unchanged after 2 h induction (Figures 4Q–4S). However, *plt2/35S::PLT2:GR* roots failed to expand the competence zone (Figures 4P and 4S), indicating that endogenous PLT2 upregulation after PLT2 activation is required for expansion of the competence zone.

It is important to note that *PLT2-GR* fusion used in this study is functional, as *35S::PLT2:GR* seedlings displayed larger meristem (Figures 5A and 5B) and ectopic root from shoot tip (Figures 5C and 5D), as reported earlier (Galinha et al., 2007), and when *PLT2-GR* is driven under *PLT3* promoter, it triggered LR outgrowth in *plt3plt5plt7* mutant (Figures 5E–5G). We next examined if PLT2 activates root tip regeneration in a dosage-dependent manner. In *Col/35S::PLT2:GR*, the acquired efficiency to regenerate beyond the competence zone after resection was highest 2 h after induction, intermediate 16 h after induction, and absent 48 h after induction (Figure 6A). Forty-eight hours after induction, roots failed to regenerate even when resected well within the competence zone, leaving only residual cell proliferation at the cut ends (Figures 6B and 6C), while WT control roots regenerated fully grown new meristem (Figure 6D). Though the meristem size was increased (Figures 5H and 5I), there were fewer cells with CYCB1;1::GFP expression, which extended till early differentiation zone (Figures 6E and 6F). The low CYCB1;1::GFP expression frequency persisted even during early days after resection, post 48 h induction (Figures S5A and S5B). This suggests that forced expression of PLT2 for a prolonged duration of 48 h impedes the restoration of root tip and affects the cell division status of the root. Inability of root tip regeneration was coupled with downregulation of PLT2::YFP at 48 h after induction (Figures 6G and 6H). When these roots were resected beyond the competence zone, they did not show any changes in PLT2::YFP levels at 6 hpc, while those resected 16 h after induction showed significant upregulation as well as root tip regeneration (Figures 6A and 6I–6L). The upregulation of PLT2::YFP noticed in the roots resected 16 h after induction was maintained even after removal of Dex. Taken together, our data suggest that PLT2 activates root tip

(P–R) Confocal images of non-regenerating root, resected beyond the competence zone (P), and regenerating roots, resected within competence zone (Q and R), of *plt2/35S::PLT2:GR* after 2 h Dex induction (P and Q) and no induction (R). After resection, the roots were transferred onto Dex-free medium. Dashed lines in the uncut images mark the cut site, which is at the elongation zone in (P), and the distance of cut is indicated in (Q) and (R). Scale bars: 50 μm.

(S) Regeneration frequency in *Col/35S::PLT2:GR* and *plt2/35S::PLT2:GR*, when resected within the competence zone and beyond the competence zone after 2 h Dex induction; error bar represents SEM.

See also Figures S2–S4.

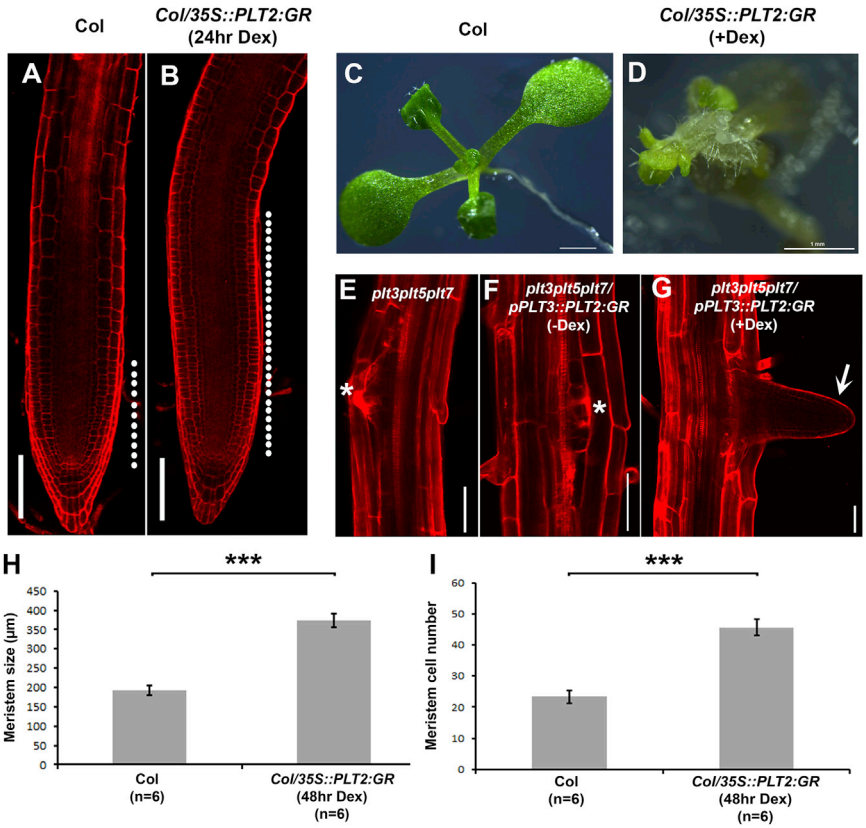

Figure 5. Induction of PLT2 Expression Causes Increase in Meristem Length, Development of Root from Shoot Apex, and Outgrowth of Lateral Root

(A and B) Confocal images of root of Col (A) and *Col/35S::PLT2:GR* subjected to Dex induction for 24 h (B). Dotted lines drawn to the right of the image mark the length of meristem. Scale bars: 100 μm.

(C and D) Shoot apex of Col (C) and *Col/35S::PLT2:GR* subjected to continuous Dex induction (D). Ectopic root with root hairs originating from shoot apex can be seen in (D). Scale bars: 1 mm.

(E–G) Confocal images of root of *plt3plt5plt7* mutant (E) and *plt3plt5plt7/pPLT3::PLT2:GR*, before (F) and after (G) Dex induction. Asterisks in (E) and (F) mark the defective lateral root primordium. Arrow in (G) marks the outgrown lateral root. Scale bars: 50 μm.

(H) Significant difference in the root meristem size, measured in micrometers, between Col and 48 h Dex-treated *Col/35S::PLT2:GR* (Welch two-sample t test: degrees of freedom [df] = 9.0303, t = −8.0874, p = 1.989×10^{-5}). At 48 h Dex induction, though the level of CYCB1;1::GFP expression is low, CYCB1;1::GFP foci extend till the differentiation zone in the roots of *Col/35S::PLT2:GR*, and the cells are dividing till the differentiation zone. In these larger meristems, meristem size is quantified by measuring the distance between QC cells and shootward boundary of the meristem, which is determined on the basis of the difference in cortical cell length, as shown in Galinha et al. (2007). Asterisks illustrates the p value: ***p < 0.001. Error bars represent SEM.

(I) Significant difference in the root meristem size, measured by counting cortical cell number, between Col and 48 h Dex-treated *Col/35S::PLT2:GR* (Welch two-sample t test: df = 9.5936, t = −6.831, p = 5.597×10^{-5}). At 48 h Dex induction, though the level of CYCB1;1::GFP expression is low, CYCB1;1::GFP foci extend till differentiation zone in the roots of *Col/35S::PLT2:GR*, and the cells are dividing till the differentiation zone. In these larger meristems, cortex cells are smaller in size, and at a few places, it is very difficult to distinguish these cells from other dividing meristematic cells. The root meristem size is quantified by counting the number of cortex cells extending from QC to the first elongated cortex cell (Perilli and Sabatini, 2010). Asterisks illustrates the p value: ***p < 0.001. Error bars represent SEM.

regeneration in a dosage-dependent manner and that sustained exposure to nuclear PLT2, beyond a threshold, leads to reduction of regeneration potential by impairing endogenous PLT2 expression.

DISCUSSION

The remarkable regeneration potential of plant cells is widely mentioned in biology textbooks, with reference to agriculturally important *in vitro* plant propagation protocols (Murashige, 1979; Vasil and Thorpe, 1994). Interestingly, this *in vitro* potential is correlated with the activity of meristem or stem cell maintenance genes or embryo-specific genes (Deng et al., 2009; Gaj et al., 2005; Gordon et al., 2007; Zuo et al., 2002). It is likely that the competence to regenerate is related to the capacity of some plant tissues to regenerate tissue structure after wounding. However, the factors that determine *in vivo* regeneration competence are not yet well understood. In the root tip, meristematic cells harbor the regeneration potential, while cells in the differentiated part of the root do not (Sena et al., 2009). Therefore this system possesses a clear regeneration potential boundary. Whole-tissue and cell-level transcriptomics data suggest the activation of hormone signaling pathways and the reacquisition of a more embryonic transcriptional state in regeneration competent cells (Efroni et al., 2016). However, key factors that determine this competence boundary have hitherto not been revealed. Our study uses PLT2 to uncover that the high-expression domain of PLT proteins is necessary and sufficient for regeneration competence.

Reduced cell division in *plt1plt2* mutant and restoration of cell division in elongation zone upon PLT overexpression has been described during normal development of the root meristem as well as in response to ablation of specific cell types in the root meristem (Xu et al., 2006; Galinha et al., 2007; Marhava et al., 2019). On the basis of these studies, one would expect that the ensuing longer meristem after prolonged PLT2 overexpression restores the missing root tip upon resection. Surprisingly, this is not the case. Only residual cell proliferation was observed upon severing these longer meristems, but no repatterning and restoration of the missing root tip. Thus, our study separates the local cell proliferation response from regeneration of a complete organ. Strikingly, the ability of PLT2 to promote regeneration of the root tip upon root tip resection is critically dependent on upregulation of endogenous PLT2. Several lines of evidence suggest that maintenance of upregulated expression of endogenous *PLT2* upon resection is instrumental for root tip

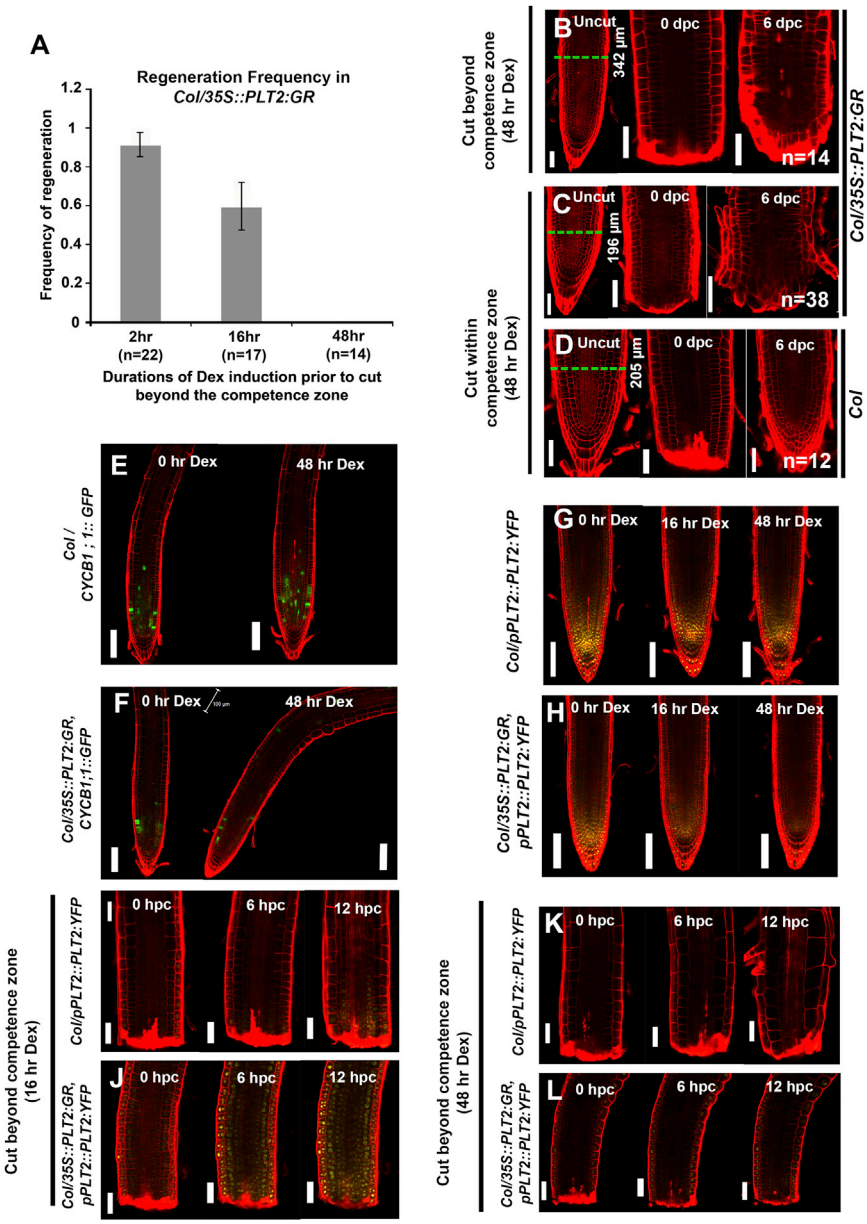

Figure 6. Threshold Level of PLT2 Is Important for Organ Regeneration

(A) Regeneration frequency in *Col/35S::PLT2:GR*, when resected beyond the competence zone after different durations of Dex induction; error bars represent SEM.

(B–D) Confocal images of non-regenerating roots of *Col/35S::PLT2:GR* (B and C) and regenerating root of Col (D), resected beyond the competence zone (B) and within the competence zone (C and D). Prior to resection, the roots were subjected to Dex induction for 48 h. After resection, the roots were transferred onto Dex-free medium. In (B) and (C), at 6 dpc, some cell proliferation can be observed at the cut end, but there is no re-patterning, and the root tip fails to restore the missing part. Dashed lines in the uncut images mark the cut site, and the distance of cut is indicated. Scale bars: 50 μm.

(E and F) Confocal images of single root of *Col/CYCB1;1::GFP* (E) and *Col/35S::PLT2:GR, CYCB1;1::GFP* (F), expressing CYCB1;1::GFP, before and after 48 h Dex induction. Scale bars: 100 μm.

(G and H) Confocal images of single root of *Col/pPLT2::PLT2:YFP* (G) and *Col/35S::PLT2:GR, pPLT2::PLT2:YFP* (H), expressing pPLT2::PLT2:YFP, before and at 16 and 48 hr Dex induction. Scale bars: 100 μm.

(I and J) Confocal images of single root of *Col/pPLT2::PLT2:YFP* (I) and *Col/35S::PLT2:GR, pPLT2::PLT2:YFP* (J), expressing pPLT2::PLT2:YFP, during first 12 h post-resection beyond the competence zone. Prior to resection, the roots were subjected to Dex induction for 16 h. After resection, the roots were transferred onto Dex-free medium. Scale bars: 50 μm.

(K and L) Confocal images of single root of *Col/pPLT2::PLT2:YFP* (K) and *Col/35S::PLT2:GR, pPLT2::PLT2:YFP* (L), expressing pPLT2::PLT2:YFP, during first 12 h post-resection beyond the competence zone. Prior to resection, the roots were subjected to Dex induction for 48 h. After resection, the roots were transferred onto Dex-free medium. Scale bars: 50 μm.

See also Figure S5.

regeneration: (1) *plt2* mutants fail to regenerate when severed beyond the competence zone, even in the presence of ectopic PLT2 overexpression. (2) Root tip regeneration is severely impaired in regions beyond the competence zone where endogenous *PLT2* is not upregulated to the optimal level, as seen within the competence zone upon resection. (3) After 48 h of Dex induction, endogenous *PLT2* is upregulated neither within the competence zone nor beyond the competence zone in *35S::PLT2:GR* root tip, and upon resection the root tip failed to regenerate.

Transient overexpression of PLT2 could not only expand the competence zone up to the differentiation zone by activating the expression of endogenous *PLT2* but also uncoupled expansion of the competence zone from changes in organ size. Our work provides a molecular basis underlying the expansion of the competence zone and suggests the operation of an auto-regulatory loop of PLT2, which plays a critical role in maintaining the optimal levels of endogenous *PLT2* expression. Future studies should test whether the nature of the *PLT2* auto-regulatory loop is direct or indirect. In either case, optimal levels of PLT2 are required for positive autoregulation. A recent finding of PLT acting in multi-protein complex with other accessory transcription factors to turn on their targets further underscores the importance of its threshold in controlling organ restoration after resection (Shimotohno et al., 2018). Sustained high levels of PLT2, beyond a threshold, are likely to disrupt the stoichiometry of multi-protein complex, leading to collapse of auto-regulatory loop and deregulation of downstream target genes essential for root tip regeneration. PLT2 has been shown to act in a

threshold-dependent way to define developmental zones during root development (Galinha et al., 2007; Mähönen et al., 2014), and now we show that the dosage of this gradient-expressed PLT2 determines the competence level for regeneration. We demonstrate this threshold in tissues in which PLT2 is or has been made limiting, and it is likely that the complete functionally redundant PLT/BBM gene clade with its large overlapping set of target genes (Santuari et al., 2016) determines this threshold under physiological circumstances.

Our data indicate that regeneration competence does not depend on size of the organ but instead depends on the level of PLT2 expression, which is indirectly linked to organ size (Mähönen et al., 2014). Other PLTs, which share the ability to cause expansion of meristem with PLT2 (Mähönen et al., 2014), may also contribute to regeneration competence. Among them, PLT1 appears to be a potential candidate, because of its higher expression level and longer expression domain, similar to PLT2 (Shimotohno et al., 2018). Likely, several other factors are also critical for the potential to regenerate. Previous works have shown that ERF115-PAT1 heterodimeric transcription factor complex and its downstream target, WIND1, play an important role during root tip regeneration by the stimulation of replenishing cell divisions (Heyman et al., 2016; Ikeuchi et al., 2019). In addition, reduction of retinoblastoma-related (RBR) levels enhances the effect of PLT2 overexpression and leads to re-entry of differentiated cells into organ formation programs (Galinha et al., 2007). Interestingly, jasmonate-triggered activation of root SCN through the RBR-SCR network and stress response protein ERF115 leads to restoration of root tip lost after resection (Zhou et al., 2019). As the gradient expression of PLT/BBM proteins imposes a boundary on the regeneration potential of cells in roots, it will be interesting to find out whether this holds true in the root context only or whether regeneration potential in meristems and embryos is also bounded by domains where these genes are highly expressed.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.08.099.

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AUTHOR CONTRIBUTIONS

K.D. and K.P. conceived and designed the research. K.D. performed all the experiments. M.V.R. and A.V.M. helped K.D. with the switchable stem cell system, inducible PLT2 RNAi silencing system, and 35S::PLT2:GR experiments. K.R. performed statistical analysis related to protein quantification and revised figures. K.D. and K.P. analyzed the data with input from A.P.M., V.W., and B.S. K.D., K.P., and B.S. wrote the manuscript. A.K., A.P.M., V.W., and B.S. contributed important reagents.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Propidium iodide	Sigma	Cat#P4170
β-Estradiol	Sigma	Cat#E2758
Dexamethasone-Water soluble	Sigma	Cat#D2915
Spectrum Plant Total RNA kit	Sigma	Cat#STRN50
High Capacity cDNA Reverse Transcription kit	Applied Biosystems	Cat#4368814
Experimental Models: Organisms/Strains		
Arabidopsis: plt2	Aida et al., 2004	N/A
Arabidopsis: plt1plt2	Aida et al., 2004	N/A
Arabidopsis: plt3plt5plt7	Prasad et al., 2011	N/A
Arabidopsis: plt3plt5plt7/pPLT3::PLT2:GR	Kareem et al., 2015	N/A
Arabidopsis: R2D2	Liao et al., 2015	N/A
Arabidopsis: Col/pPLT2::PLT2:YFP	Mähönen et al., 2014	N/A
Arabidopsis: Col/pPLT2::CYCB1;1:YFP	Mähönen et al., 2014	N/A
Arabidopsis: plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi	Mähönen et al., 2014	N/A
Arabidopsis: plt1plt2/pCYP79B3::PLT2:YFP	Mähönen et al., 2014	N/A
Arabidopsis: Col/CYCB1;1::GFP	Mähönen et al., 2014	N/A
Arabidopsis: plt1plt2/pCYP79B3::PLT2:CFP	This paper	N/A
Arabidopsis: Col/DR5::3XVENUS-N7, pPIN1::PIN1:GFP	Pinon et al., 2013	N/A
Arabidopsis: plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP	This paper	N/A
Arabidopsis: Col/35S::PLT2:GR, pPLT2::PLT2:YFP	This paper	N/A
Arabidopsis: Col/35S::PLT2:GR, CYCB1;1::GFP	This paper	N/A
Arabidopsis: plt2/35S::PLT2:GR	This paper	N/A
Arabidopsis: Col/35S::PLT2:GR	This paper	N/A
Oligonucleotides		
3′ UTR region of PLT2: *qRT FP- GGTAGGGTATGGAATAATTAGC*	This paper	N/A
3′ UTR region of PLT2: *qRT RP- CCTAAAAAGACTAACCCT CGAG*	This paper	N/A
Recombinant DNA		
35S::PLT2:GR	This paper	N/A

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Kalika Prasad (kalika@iisertvm.ac.in).

This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant material

Arabidopsis thaliana plants of Columbia ecotype (Col-0), Columbia-Utrecht (Col-utr) ecotype and Wassilewskija ecotype (Ws) were used in this study. *Arabidopsis* mutants, *plt2*, *plt1plt2*, *plt3plt5plt7* have been described previously (Aida et al., 2004; Prasad et al., 2011). Transgenic lines, *plt3plt5plt7/pPLT3::PLT2:GR, R2D2, Col/pPLT2::PLT2:YFP, Col/pPLT2::CYCB1;1:YFP, plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi, plt1plt2/pCYP79B3::PLT2:YFP, Col/CYCB1;1::GFP* have been described previously

(Kareem et al., 2015; Liao et al., 2015; Mähönen et al., 2014). Transgenic line, *plt1plt2/pCYP79B3::PLT2:CFP,* used in this study, has *pCYP79B3::PLT2:CFP* construct, that was made in exactly the same way as *pCYP79B3::PLT2:YFP* of *plt1plt2/pCYP79B3::PLT2:YFP.* The only difference is that the reporter used is CFP instead of YFP. DR5::VENUS expression was examined in *Col/DR5::3XVENUS-N7, pPIN1::PIN1:GFP* line, which has been described previously (Pinon et al., 2013). Though a double marker line of DR5::3XVENUS-N7 and pPIN1::PIN1:GFP was used, the expression of only DR5::VENUS, but not of pPIN1::PIN1:GFP, was recorded. *plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP* was generated by crossing *plt3plt5plt7/pPLT3::PLT2:GR* (Kareem et al., 2015) with *plt3plt5plt7/pSCR::H2B:YFP* (Kareem et al., 2015). *Col/35S::PLT2:GR, pPLT2::PLT2:YFP* was generated by crossing *Col/35S::PLT2:GR* with *Col/pPLT2::PLT2:YFP* (Mähönen et al., 2014). *Col/35S::PLT2:GR, CYCB1;1::GFP* was generated by crossing *Col/35S::PLT2:GR* with *Col/CYCB1;1::GFP* (Mähönen et al., 2014). *plt2/35S::PLT2:GR* and *Col/35S::PLT2:GR* were generated by transforming *plt2 mutant* (Aida et al., 2004) and Col-0 respectively, with *35S::PLT2:GR* construct, by floral dip method (Clough and Bent, 1998).

Growth conditions

Seeds of *A.thaliana* were surface sterilized with 70% ethanol and 20% bleach, followed by 7 washes with sterile distilled water. Seeds were plated on half-strength Murashige-Skoog (MS) medium (pH 5.8) and grown vertically under a long-day regime (16hr light/8hr darkness), at 22°C and 70% relative humidity. Seedlings at 4 day post germination (dpg) or 5dpg were used for resection.

METHOD DETAILS

Plasmid construction

35S::PLT2:GR was constructed by cloning the genomic sequence of *PLT2* (Mähönen et al., 2014), which was fused in-frame to rat glucocorticoid receptor (GR) (Aoyama and Chua, 1997), under *CaMV* 35S promoter, in pCAMBIA 1300 binary based destination vector, through Gateway recombination.

Estradiol and Dexamethasone inductions

In case of *plt1plt2+/−plt3plt4/pPLT2::PLT2:YFP, pG10-90::PLT2 RNAi(F4 generation),* 3dpg old seedlings were transferred to MS medium supplemented with 5μM Estradiol and incubated for 47hr, before resection.

In case of *Col/35S::PLT2:GR,* 3dpg old (for 16hr and 48hr Dex induction) or 4dpg old (for 2hr Dex induction) seedlings were transferred to MS medium supplemented with 20μM Dex and incubated for appropriate duration before resection. After resection, seedlings were transferred to Dex free MS medium.

In case of *plt3plt5plt7/pPLT3::PLT2:GR, pSCR::H2B:YFP,* 4dpg old seedlings were transferred to MS medium supplemented with 20μM Dex and left for 8hr, before they were re-transferred to Dex free MS medium. The 8hr Dex induction was sufficient to trigger LR formation later. After removal of Dex induction the emerged LRs were examined for loss of SCR expression in QC and surrounding cells and the ones at the right stage, were chosen for resection. In case of control, the seedlings were subjected to continuous Dex induction. Only young LRs were chosen for resection.

Root tip resection and microscopy

The seedlings were placed on a sterile glass slide, over a few drops of sterile water and their root tips were resected by hand, using a sterile 1ml syringe (Dispovan), under Zeiss Axio Scope.A1 microscope. Resections were performed at the root tip in the region encompassing from QC to differentiation zone. The boundary of the competence zone is defined as the largest distance from the fourth tier of columella cells, leading to regeneration post resection. In order to measure the position of resection, immediately after resection, the image of the detached part of the root tip was captured. Then in the captured image, the distance between the cut end and fourth tier of columella cells was measured in micrometer. Due to the damage caused upon resection, there is possibility for error in the measurement, which could range from 30μm to 70μm. For any given experiment where root tip regeneration frequency has been quantified between two or among three different genetic backgrounds, only those samples were taken for analysis which had similar extent of damages at the cut ends after resection in both or all the three genetic backgrounds subjected for comparison. Very high damage at the cut site, which is far above 70μm, is a technical failure of resection and it sometimes results in no regeneration of even regeneration-competent root tips post resection. In all the resection experiments presented in the manuscript, damage caused at the cut site, upon resection, was maintained at minimal. After resection, seedlings were transferred back to MS medium. Meristem size was calculated as the distance between QC cells and shootward boundary of the meristem, which is determined based on the difference in cortical cell length (Galinha et al., 2007).

For confocal imaging, the resected seedlings were placed over a sterile glass slide and mounted with filter-sterilized, 20μg/ml propidium iodide (PI). They were imaged using Leica TCS SP5 II laser scanning microscope and then washed with sterile water, before they were re-transferred to MS medium. Nomarski (DIC) images were taken using Zeiss Axio Scope.A1 microscope. Images were compiled using Adobe Photoshop CS6.

Root tip regeneration was confirmed based on growth and morphology of the root tip at 5dpc.

qRT-PCR Analysis

Total RNA was extracted from Col-0 and *Col/35S::PLT2:GR* root tips (*encompassing columella, meristem, transition zone and elongation zone*), which were subjected to 8hr Dex induction, by using Spectrum Plant Total RNA kit (Sigma) and on column DNase treatment was performed as per the manufacturer's guidelines. The cDNA was synthesized from 1μg total RNA using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). qRT-PCR was performed using 3 μl of one-tenth diluted cDNA in a total reaction volume of 25μl and the primers used were designed against 3′ UTR region of PLT2 (*qRT FP- GGTAGGGTATGGAATAA TTAGC, qRT RP- CCTAAAAAGACTAACCCTCGAG*). Obtained results were normalized against *ACTIN2* (*ACT2*) expression. The relative gene expression was represented as fold-change value by calculating $-\Delta\Delta C^T$. Two independent biological replicates were used and each biological replicate was tested in technical triplicates.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of PLT2 protein intensity was done using 'Quantify' tool in LAS AF Lite software. The region chosen for quantification is different in different quantification graphs and is shown in the figures. The cell files present in the region chosen for quantification are mentioned in the figure legends.

Quantification of meristem size was done, as mentioned in the figure legend.

For all the statistical analysis, details of the type of statistical test used, P value and other statistical parameters are mentioned in the figure legends. For multiple comparisons, ANOVA followed by Tukey's Honestly Significant Difference (HSD) test was performed in some cases. In some other cases, Kruskal-Wallis test followed by Mann-Whitney U test or just Mann-Whitney U test or Welch two sample *t* test was performed. 'n' represents the sample size. Error bars represent either standard deviation or standard error of mean, as mentioned in the figure legends. Asterisks illustrate the P value, as mentioned in the figure legends.

DATA AND CODE AVAILABILITY

This study did not generate/analyze any datasets/code.