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Current Opinion in Plant Biology

Shoot regeneration: a journey from acquisition of competence to completion

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Plants display an extraordinary ability to regenerate complete shoot systems from a tissue fragment or even from a single cell. Upregulation of the determinants of pluripotency during a precise window of time in response to external inductive cues is a key decisive factor for shoot regeneration. A burst of recent studies has begun to provide an understanding of signaling molecules that are instrumental in the making of the regenerative mass, as well as the developmental regulators that are seminal in shaping the pluripotent state. Here, we discuss how signaling molecules, waves of mutually exclusive stem cell regulators and epigenetic modifiers could contribute to cellular heterogeneity in an island of regenerative mass, thus leading to *de novo* shoot regeneration.

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Current Opinion in Plant Biology 2018, **41**:23–31

This review comes from a themed issue on **Growth and development**

Edited by **Gwyneth Ingram** and **Ari Pekka Mähönen**

For a complete overview see the Issue and the Editorial

Available online 30th August 2017

http://dx.doi.org/10.1016/j.pbi.2017.08.001

Introduction

A zygote exemplifies a single cell capable of giving rise to a complete organism. However, plants are among the rare organisms known to possess the extraordinary capacity of awakening the totipotency that lies dormant in a few selected cells of the adult body [1•,2,3]. The current scale of plant regeneration studies, and commercial applications resulting from them, arises from our understanding of the crucial role played by two key plant hormones, auxin and cytokinin, in modulating *de novo* plant regeneration [4]. *De novo* organogenesis [5,6] and somatic embryogenesis [7] are strictly hormone-induced and hormone-controlled processes that are almost impossible without these external cues. External inductive cues of this nature, which are sufficient for cellular reprogramming to give rise to complete organism from a tissue fragment, are limited to the plant kingdom (Figure 1) [3,8]. Over the years, several studies of the loss of function of key plant specific transcription factors and the consequences of their forced expression during *de novo* shoot regeneration have permitted the unearthing of the underlying molecular mechanisms [1•,9,10••,11,12•,13,14]. In this review, we highlight how recent studies into *de novo* shoot regeneration have begun to reveal the fundamental cellular and molecular events in real time, and how this has generated renewed interest in novel areas of reprogramming [15], where a number of key questions yet remain unsolved.

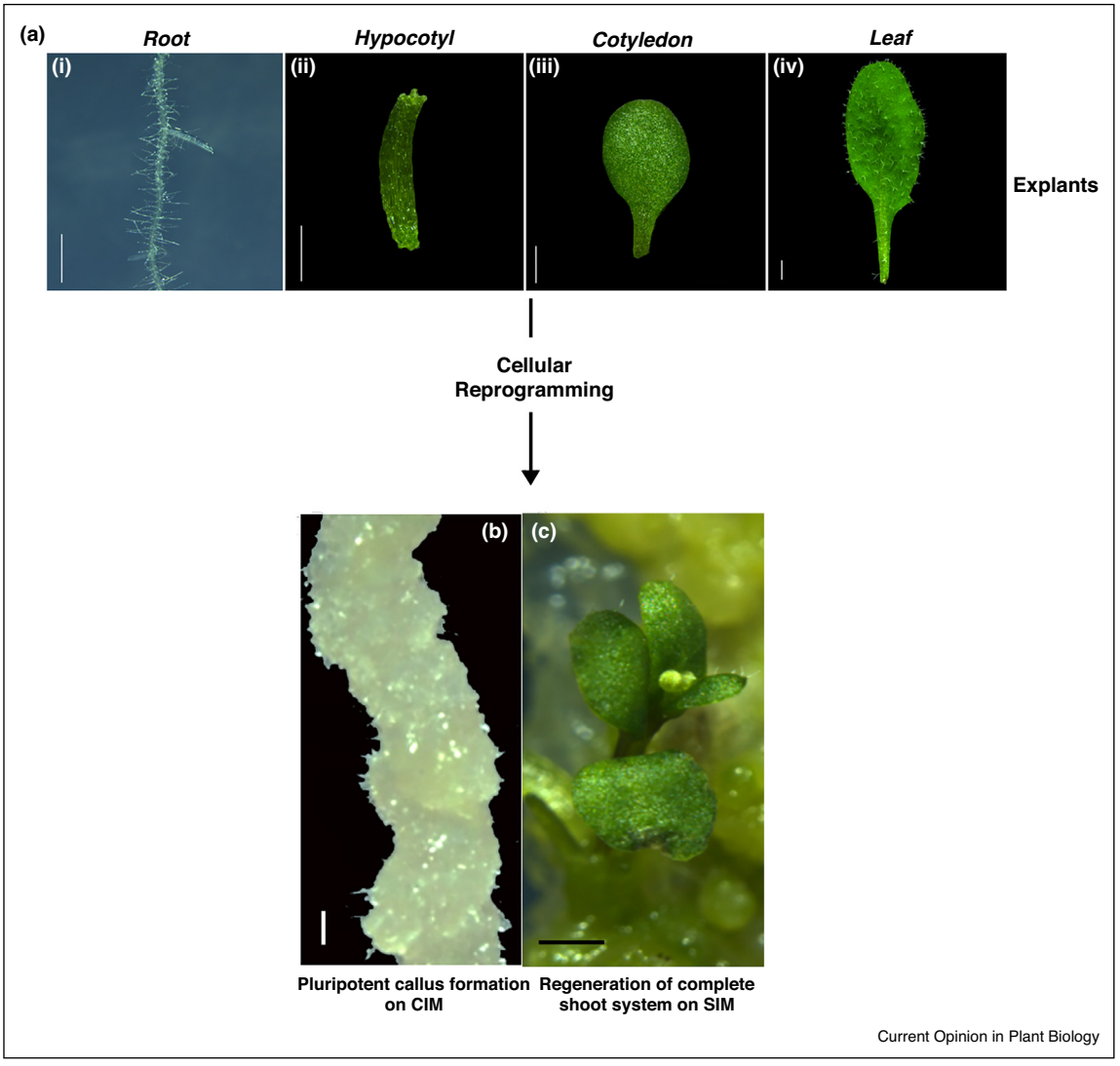
Modes of regeneration

Until now, the most investigated and best understood mode of shoot regeneration is callus mediated regeneration using a two step protocol or a modified version of this protocol [5]. Indirect *de novo* shoot regeneration involves two phases of incubation: first callus induction on an auxin rich medium, followed by shoot organogenesis on a cytokinin rich medium [10••,16–19]. The regenerative potential of explants used for callus induction largely depends on their developmental stages [18,20] and origins [5,21]. In contrast, direct shoot organogenesis involves the incubation of suitable explants on cytokinin containing media, with or without prior auxin priming [10••,16,22,23••,24]. Conversion of lateral root primordia (LRP) to shoots is an example of direct regeneration. The central role of each hormone, in moulding the plasticity of plant cells, is demonstrated by the critical importance of either auxin or cytokinin during the different phases of incubation during both modes of regeneration.

Acquisition of competence to regenerate shoot in response to relative abundance of plant hormones

The auxin to cytokinin ratio in the culture medium is key to the development of a pluripotent callus [16,25••]. Several lines of evidence demonstrate an irreplaceable role for high auxin concentration in LRP initiation [26] as well as in pluripotent callus formation [27]. The organogenetic potential and the molecular characteristics of a callus induced on a cytokinin rich callus induction medium (CIM) is markedly different from a callus induced on auxin rich CIM. High concentrations of cytokinin induces phloem pericycle cell divisions in

Figure 1



Arabidopsis explants undergoing cellular reprogramming to regenerate complete shoot systems. **(a)** Explants used for *de novo* shoot regeneration: (i) root, (ii) hypocotyl, (iii) cotyledon, and (iv) leaf. **(b)** Pluripotent callus formation from explant on callus induction medium (CIM). **(c)** Regeneration of complete shoot system from callus on shoot induction medium (SIM). Scale bars in (a.ii and a.iii) represent 0.5 mm and 1 mm in the rest of the images. Image (c) is reprinted from Kareem *et al*. [10••] with permission from Cell Press. License number: 4158680029029.

contrast to the xylem pole pericycle divisions induced on auxin rich media [16]. Key root trait determinants, essential for the establishment of pluripotency (see the section below), are dramatically downregulated in cytokinin rich CIM and such callus fails to regenerate shoots [16,25••]. These studies suggest the sole use of cytokinin in CIM is not sufficient to drive the downstream molecular changes and cell divisions required to establish the pluripotency and thus the ability to regenerate shoots. During direct regeneration, auxin rich LRP are competent to make shoots when exposed to high cytokinins [10••,16,23••,24], further supporting the role of high auxin in establishing the pluripotency necessary to regenerate shoots.

A root developmental pathway is important for callus formation

Initial incubation on CIM prompts the pool of reprogrammable adult stem cells constituted by xylem-pole pericycle cells in roots, and pericycle-like cells in aerial explants, to undergo rapid division in response to exogenously applied auxin [25••,28,29]. These cell divisions and ensuing molecular changes during CIM pre-incubation play a critical role in the acquisition of competence for shoot induction [10••,16,17,19,25••,29]. As these cells proliferate, the expression of *J0121* [30], a marker for differentiated pericycle cells, becomes diffuse and disappears from the callus [17,25••,29]. The disappearance

of *J012* expression has been correlated with the higher potential to form callus, as in the case of the *callus formation related 1(cfr1) mutant* [31•]. *J0121* has an intermediate expression level in wild type callus and persists in *solitary-root (slr/iaa14)* mutants [32] which are defective in callus formation [31•]. Loss of *J0121* is not a mark of dedifferentiation during callus formation, but rather appears to mark the acquisition of a root identity by callus cells [17,25••,31•]. The root identity of the callus is supported by similarities between the morphology, cellular organization and gene expression profiles of roots and callus. Many root tissue markers such as *WUSCHEL-related homeobox 5 (WOX5)*, *SHORT-ROOT* (*SHR*), *SCARECROW* (*SCR*), *PLETHORA 1* (*PLT1)*, *PLT2*, *PIN-FORMED1* (*PIN1*), the quiescent center marker, *QC25*, *ROOT-CLAVATA HOMOLOG 1* (*RCH1*) and *GLABRA2* (*GL2)* are expressed in callus irrespective of the origin of the explants [10••,16,25••]. The significance of the systematic triggering of a root development pathway during callus formation has been further emphasised by genetic studies which demonstrate reduction or elimination of callus formation in the *aberrant lateral root formation 4* (*alf4*) mutant [25••], which is defective in lateral root initiation [33]. Interestingly, signaling molecules such as very long chain fatty acids restrict the *ALF4* mediated callus forming activity of pericycle cells [31•]. The crucial role played by *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)* transcription factors in lateral root development as well as in callus induction also reinforces the significance of the root development pathway in callus formation [34–36]. Thus, morphological and molecular similarities and genetic studies highlight the commonalities between callus formation and lateral root initiation suggesting that root-like traits are likely to influence the regeneration potential of callus.

To be or not to be competent?

The significance of root traits in conferring regenerative potential to the callus derived from all explants, irrespective of their origin, has recently been unraveled by studying the molecular and cellular phenotypes of the *plt3, 5-2, 7* triple mutant in real time [10••]. *PLT* genes encode plant specific double AP2 domain-containing transcription factors [37]. *plt3,5-2,7* mutant [38,39] callus was shown to lose key root trait determinants and the ability to regenerate any shoot progenitors [10••]. Root stem cell regulators are transcriptionally regulated by *PLT3*, *PLT5* and *PLT7* and play an essential role in conferring pluripotency and thus competence to make shoot progenitors. Subsequent over-expression of organ boundary gene *CUP-SHAPED COTYLEDON 2* (*CUC2*), which is also regulated by the *PLT* genes, helps in the successful outgrowth of the shoot from the regenerative foci. This study thus reveals a two step mechanism which appears to be valid for all explant tissues [10••]. The competence to regenerate and the timely divergence from the initial lateral root development pathway are also essential criteria for direct conversion of LRP to shoots. During direct shoot regeneration, LRP at neither very early stages nor very late stages are capable of conversion [22,23••]. The intermediate stage, competent, LRP contain a group of cells in the transient root stem cell niche that expresses *WOX5*, rapidly followed by the expression of shoot specific genes like *WUSCHEL* (*WUS)*, *CLAVATA 3* (*CLV3*) and *SHOOT MERISTEMLESS* (*STM*) when these LRP are incubated on cytokinin media [23••]. The initial expression of root stem cell regulators in LRP, together with their genetic necessity for conversion of LRP to shoot demonstrate that transient root traits are essential in pluripotent founder cells for cellular reprogramming towards shoot fate [10••,23••].

Wounding, inevitable signaling in priming the regeneration

Wounding is an inevitable process during regeneration as the formation of protuberances on CIM as well as the outgrowth of competent LRPs is characterized by forceful protrusion of these developing structures through non-converted cells. The consequent loss of contact between the adjacent differentiated cells is likely to trigger the wound signaling pathway. Interestingly, over-expression of the wound induced transcription factor, *WOUND INDUCED DEDIFFERENTIATION 1* (*WIND1*) followed by prolonged incubation on cytokinin rich medium can bypass the need for external application of artificial auxin on explants [12•]. Recent findings piece the puzzle together by providing insights into how wounding-induced signaling can promote *de novo* shoot regeneration. The *WIND1* transcription factor directly activates *ENHANCER OF SHOOT REGENERATION 1* (*ESR1*) to promote *CUP SHAPED COTYLEDON 1 (CUC1)*-mediated shoot regeneration [40•]. However the question of what establishes the pluripotency in wound induced callus remains. Are wound induced signals or transient *WIND1* overexpression during callus formation sufficient to establish pluripotency and thus competence to regenerate, in addition to *ESR1*-mediated shoot promoting activity? It is important to note that key root cell fate determinants do not display appreciable up-regulation in *WIND1* overexpressing callus as compared to callus-induced by wounding together with external auxin applications [12•]. This raises the question of whether other wound induced signals can substitute for the necessity for root cell fate determinants in conferring the pluripotent state to callus? This does not appear to be the case, as external inductive cues involving wounding in addition to auxin, fails to trigger any sign of shoot regeneration in mutants defective in lateral root cell fate determinants [10••]. Callus harbouring an artificial repressive form of *WIND1* express nearly normal levels of root cell fate determinants and does regenerate shoots, though only occasionally [40•]. Proof of the absolute necessity for *WIND1* to establish pluripotency requires clean genetics involving the cumulative loss of function of *WIND1* and

related redundant genes. Although both these and other studies are needed to gain deeper insights, the possibility that wound induced signals act together with root cell fate determinants to establish the pluripotent state, and the indispensible role of root stem cell regulators in shoot regeneration, remain central elements in our current view of this enigmatic process.

Waves of expression of distinct sets of stem cell regulators are key to progressive shoot regeneration

Regeneration is a constant tug-of-war between the root and shoot identities in response to external inductive cues. Depending on the root *vs.* shoot inductive cues, the preponderance of either root or shoot factors will drive the process along specific developmental pathways. The final phase of indirect *de novo* regeneration is the transfer of competent calli to Shoot Inducing Medium (SIM). This triggers rapid changes in morphological features and molecular markers [19]. Calli incubated on SIM show faster and unorganised proliferation outside of the central region of the root explants [16]. Another observable feature which may be easily mistaken for a sign of shoot regeneration is the greening of the calli, which has been attributed to the maturation of chlorophyll promoted by cytokinin [41,42•]. The epidermal hairs which are prominent on CIM and at early stages of SIM incubation become less pronounced on prolonged SIM incubation. The possibility of reversal (root ↔ shoot) of cell fate at the shoot regenerating foci has not been addressed during the process of callus mediated (two step) regeneration process. However, the answer to the question of reversibility can be partially deduced by following the dynamics of cellular events, transient phases of the resetting of the cellular identity and expression patterns of key factors capable of switching cell fate in real time. It is well established that root stem cell regulators such as *PLT1*, *PLT2* (Figure 3a,e), *SCR* and root specific markers like *QC25*, and *RCH1* are rapidly down-regulated during incubation on SIM (Figure 2a) [10••,16,19]. At this juncture, the expression of shoot specific genes is neither strongly upregulated nor confined to specific domains [9,10••,16,19]. Thus, there appears to exist a narrow intermediate void phase during which a defined root or shoot identity cannot be assigned, and the callus exists in a kind of 'no man's land'. The shoot stem cell regulators, *WUS* and *STM* display an upregulated expression in a confined domain only after a few days in response to high levels of cytokinin. By this time the expression of root stem cell regulators has diminished [10••,16,19]. This lag between the peaks of the expression of two distinct sets of mutually exclusive stem cell regulators may not allow multiple rounds of reversible fate switching unlike the situation observed during direct regeneration. Such a lag between the downregulation of root stem cell regulators and upregulation of shoot stem cell regulators is not observed during direct regeneration [22,23••]. The converting LRP passes through a transient phase where it apparently possesses a dual fate (both root as well as shoot stem cell regulators are expressed) (Figure 2b) [22,23••]. During the direct conversion of LRP to shoots, cell fate can be reversibly switched multiple times during a narrow window of time. During this brief period, the cell fate of the LRP, where there is an upregulation of shoot stem cell regulators due to cytokinin treatment, can be reverted to form a root meristem by subsequent auxin treatment [23••]. Lateral root primordia cells expressing both root and shoot stem cell regulators thus appears to act as bipotent stem cells that can give rise to either root or shoot fate.

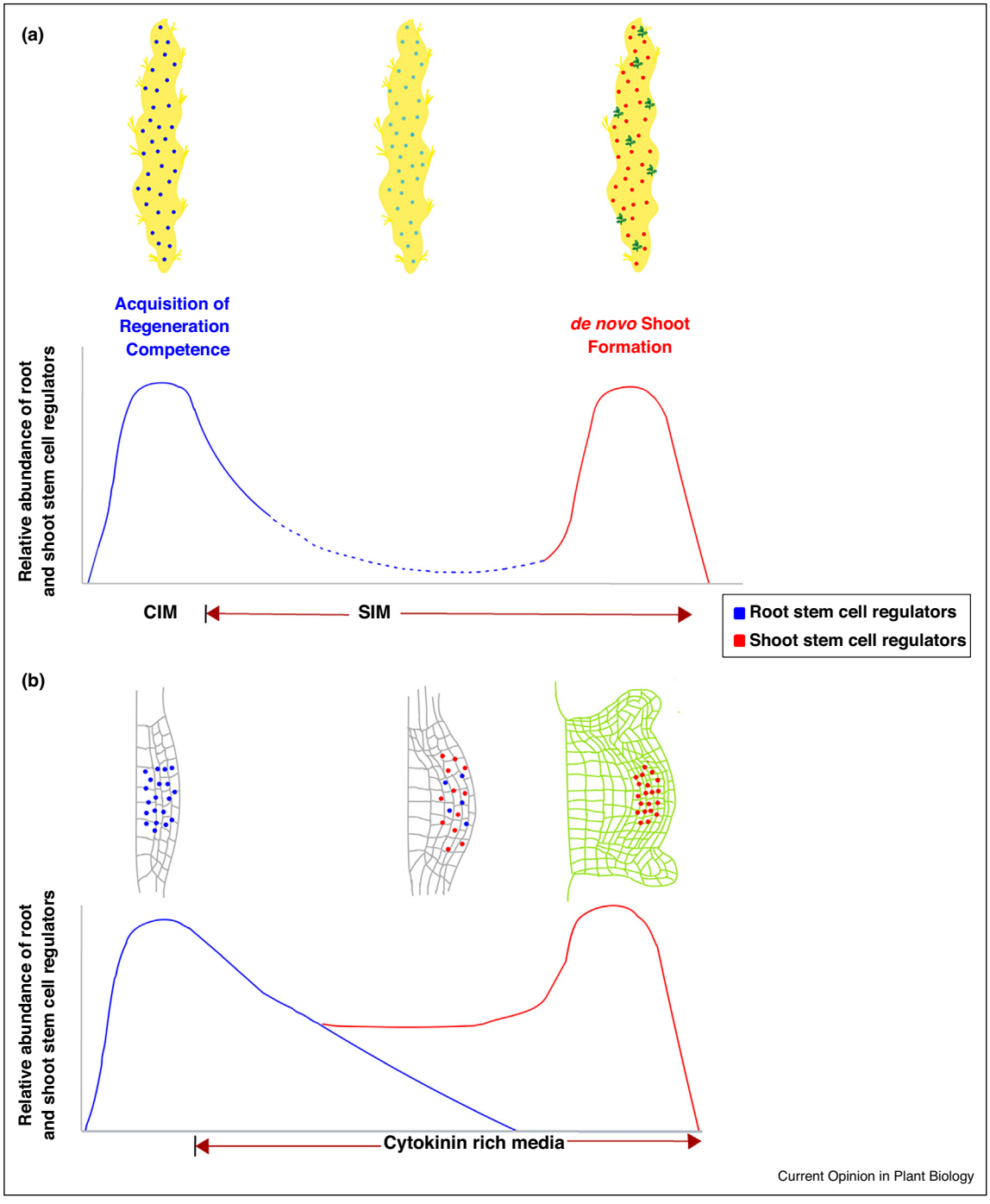
Uncoupling the intermediate developmental phases during regeneration

A central question that remains largely unanswered across the plant kingdom is whether regeneration proceeds through intermediate developmental phases and whether these phases can be uncoupled? While indications of potential intermediate phases were realized during morphological studies of regenerating callus across Arabidopsis accessions [42•], a major challenge was to uncover the cellular and molecular mechanisms that could uncouple these intermediate phases of shoot regeneration. A key step in understanding the mechanisms of regeneration was the discovery of distinct *PLT* regulated modules that uncoupled the acquisition of competence to regenerate shoot progenitors from the completion of shoot regeneration [10••]. The study involved the step by step reintroduction of root stem cell regulators and shoot promoting factors in a mutant blocked at an intermediate phase of shoot regeneration. This resulted in a sequential unblocking of the intermediate phases, thereby identifying key regulatory modules required to accomplish shoot regeneration [10••].

Cellular heterogeneity during shoot regeneration

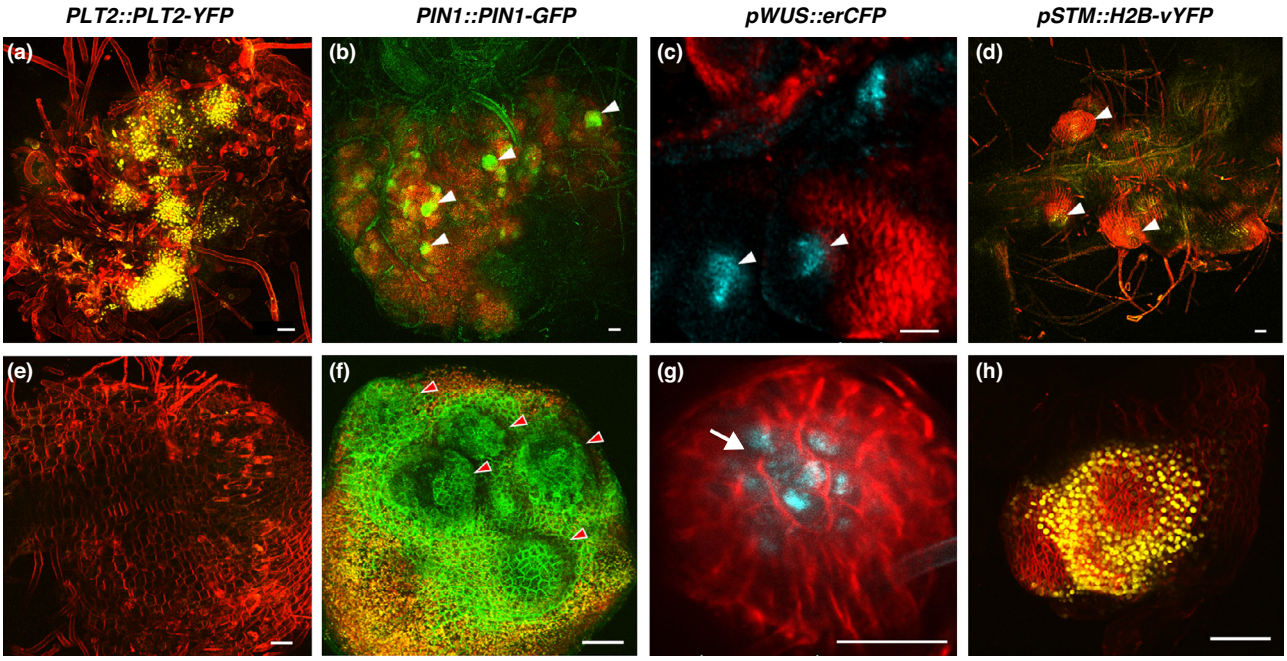
The *de novo* assembly of a shoot in an island of callus appears to follow the basic principles of self-organisation. The tightly controlled spatio-temporal order of regulatory events plays a determining role in the absence of any embryonic or postembryonic positional cues. Despite the fact that callus cells are thought to be pluripotent, not all the cells are capable of initiating the regenerating foci and thus there exists cellular heterogeneity within the callus. In fact, heterogeneity in the gene expression levels, and non-overlapping expression domains of the key shoot and root determinants highlights the considerable differences amongst the cells of regenerating calli (Figure 3) [10••,16,19]. The level of heterogeneity increases over time in culture. Even though a number of shoot regenerating foci can be initiated and produce *PIN1* marked shoot progenitors, not all of these progenitors can accomplish the regeneration of a complete shoot system. The fate of the regenerating foci and their conversion are

Figure 2



Schematic illustration representing waves of expression of root and shoot stem cell regulators during *de novo* shoot regeneration. **(a)** During callus formation (on CIM), high expression levels of root stem cell regulators is shown by the blue peak. It defines the phase of acquisition of regeneration competence. Following transfer to SIM, root stem cell regulators are down-regulated and key shoot stem cell regulators are not yet upregulated and confined to their respective domains (represented by the dotted blue line and absence of red line). Later during shoot regeneration (on SIM), the up-regulation of shoot stem cell regulators (red peak) marks the initiation of *de novo* shoot formation. **(b)** During direct shoot regeneration on cytokinin rich media the level of root stem cell regulators drops gradually as shown by the blue line. However, shoot stem cell regulators shown by red line appear before the root stem cell regulators have been lost, thereby imparting a mixed cell fate identity to the converting LRP [10••,16,19,22,23••].

Figure 3



Cellular heterogeneity in regenerating callus. Expression of root and shoot specific regulators is restricted to only few foci in the mass of callus. **(a)** Expression of *PLT2*::*PLT2-YFP* in callus on CIM. (b–d) Overviews showing the expression of *PIN1*::*PIN1-GFP* **(b)** *pWUS*::*erCFP* **(c)** and *pSTM*::*H2B-vYFP* **(d)** in regenerating shoot foci on SIM. **(e)** Down-regulation of *PLT2*::*PLT2-YFP* in callus upon shoot inductive stimuli. **(f)** *PIN1*::*PIN1-GFP* expression in a regenerated shoot meristem with organ primordia. **(g)** Confined expression of *pWUS*::*erCFP* (arrow) to the center of a shoot promeristem. **(h)** Regenerating shoot meristem displaying *pSTM*::*H2B-vYFP* expression. Red colour in (a) propidium iodide, (b and f) chlorophyll autofluorescence and (c, d, e, g, and h) FM4-64 stain. Scale bar represents 50 μm except in (g) where it represents 30 μm. White arrowheads in (b, c, and d) indicate the regenerative foci in the heterogeneous callus. Red arrowheads in (f) indicate the organ primordia. White arrow in (g) indicates *WUS* confinement in a shoot promeristem.
Image (c) is reprinted from Kareem *et al*. [10•• with permission from Cell Press. License number: 4158680029029).

apparently stochastic, and decisions are likely to be influenced by irregular callus topology that could impose growth driven mechanical tension [43], environmental factors such as contact with the culture medium, and endogenous inter-cellular variability at the level of gene expression or genome status. One of the prime factors that has been proposed to contribute to cellular and molecular heterogeneity during regeneration is underlying epigenetic variations resulting from hormone treatments and multiple rounds of cell divisions [44]. Epigenetic regulation of *WUS* [45,46], a key gene that has been shown to be involved in establishing the stem cell niche during normal [47,48] as well as *de novo* shoot development [14,19,23••,45] provides one of the examples of a mechanism by which cellular heterogeneity is generated. Deregulated epigenetic modifications at the *WUS* locus could change expression competence and generate cellular heterogeneity. The higher number of organizing centers in the (*methyltransferase 1*) *met1*, DNA hypomethylated mutant, could be a result of ectopic relaxation of repressive epigenetic control at the *WUS* locus [46]. In addition to epigenetic modifiers, a multitude of other factors, and cross talk between factors, can also contribute to the disparity in the regenerative potential between callus cells [49].

Perspective

We have begun to understand the molecular mechanisms underlying *de novo* plant regeneration. Over the years, the availability of cutting edge techniques such as single cell RNA sequencing [50••,51], time lapse imaging [22,52–54], and cell lineage analysis [55] have helped in scrutinizing developmental processes in finer detail than ever before. This has resulted in the discovery of new populations of adult stem cells [25••], the dynamics of cellular events and the assembly of regulatory interactions in real time [10••,19,23••]. Furthermore, the power of classical genetics has permitted the elucidation of how the players engaged in normal developmental pathways have been hijacked to accomplish reprogramming [10••,50••]. For example, the root regulatory network is required to establish the pluripotent state in callus and embryonic patterning gene such as *CUC2* is needed to facilitate the completion of shoot regeneration [10••,25••]. More careful

studies are indispensable for allowing clarification of the parallels between normal developmental pathways and different phases of shoot regeneration, as exemplified in the case of root regeneration, which has been shown to follow an embryo-like developmental sequence to regenerate the root tip upon resection [50••]. Unraveling each new detail enhances our understanding and appreciation of the challenges that lie ahead in understanding the complex process of regeneration. While several lines of evidence demonstrate a key role for the transient induction of root stem cell regulators in *de novo* shoot formation, both during indirect and direct [10••,23••] regeneration in *Arabidopsis*, parallel studies must be carried out in other plant species to determine if the necessity of root regulatory network in conferring pluripotency during shoot regeneration is conserved [56].

While *de novo* shoot regeneration can be exploited to answer a number of fundamental questions addressing the cellular and molecular mechanisms of reprogramming in the absence of embryonic positional cues, the discovery of new regulators capable of conferring regenerative potential to recalcitrant plant species or enhancing regeneration efficiency, can also drive innovation in green culture industries. Future studies should exploit plant regeneration as a model to study how plants have deployed kingdom-specific mechanisms to deal with intrinsic differences such as lack of cell migration, a key cellular process, extensively utilized in animal regeneration.

Acknowledgements

K.P. acknowledges the grant from Department of Biotechnology (DBT), Govt. of India and infrastructure and financial support from Indian Institute of Science Education and Research, Thiruvananthapuram (IISER-TVM). D.R. acknowledges University Grants Commission (UGC) fellowship. A.K. is supported by IISER-TVM fellowship. K.D. is a recipient of INSPIRE fellowship. S. E. acknowledges Council of Scientific and Industrial Research (CSIR) fellowship. We thank the handling editor, Gwyneth Ingram for her suggestions and inputs during the revision.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Ikeuchi M, Ogawa Y, Iwase A, Sugimoto K: **Plant regeneration: cellular origins and molecular mechanisms [Internet]**. *Development* 2016, **143**:1442-1451.
The authors provide an overview on the regeneration capacity of a variety of organisms with a comprehensive account on various modes of plant regeneration and their molecular basis.

2. Pulianmackal AJ, Kareem AV, Durgaprasad K, Trivedi ZB, Prasad K: **Competence and regulatory interactions during regeneration in plants [Internet]**. *Front Plant Sci* 2014, **5**:142.

3. Kareem A, Radhakrishnan D, Sondhi Y, Aiyaz M, Roy MV, Sugimoto K, Prasad K: **De novo assembly of plant body plan: a step ahead of Deadpool [Internet]**. *Regeneration (Oxf)* 2016, **3**:182-197.

4. Skoog F, Miller CO: **Chemical regulation of growth and organ formation in plant tissues cultured in vitro**. *Symp Soc Exp Biol* 1957, **11**:118-130.

5. Valvekens D, Montagu MV, Lijsebettens MV: **Agrobacterium tumefaciens-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection [Internet]**. *Proc Natl Acad Sci U S A* 1988, **85**:5536-5540.

6. Che P: **Gene expression programs during shoot, root, and callus development in Arabidopsis tissue culture [Internet]**. *Plant Physiol* 2006, **141**:620-637.

7. Huang B, Yeoman MM: **Somatic embryogenesis in *Arabidopsis thaliana* L. [Internet]**. In *Somatic Embryogenesis and Synthetic Seed II*. Edited by Bajaj YPS. Springer Berlin Heidelberg; 1995:371-384.

8. Birnbaum KD, Sanchez Alvarado A: **Slicing across kingdoms: regeneration in plants and animals [Internet]**. *Cell* 2008, **132**:697-710.

9. Meng W, Cheng ZJ, Sang YL, Zhang MM, Rong XF, Wang ZW, Tang YY, Zhang XS: **Type-B ARABIDOPSIS RESPONSE REGULATORs is critical to the specification of shoot stem cell niche by dual regulation of WUSCHEL**. *Plant Cell* 2017 http://dx.doi.org/10.1105/tpc.16.00640.

10. •• Kareem A, Durgaprasad K, Sugimoto K, Du Y, Pulianmackal AJ, Trivedi ZB, Abhayadev PV, Pinon V, Meyerowitz EM, Scheres B *et al.*: **PLETHORA genes control regeneration by a two-step mechanism [Internet]**. *Curr Biol* 2015, **25**:1017-1030.
The study describes a two-step mechanism of shoot regeneration applicable to all explants regardless of origin. The study was successful in uncovering distinct regulatory modules that uncoupled the acquisition of competence to regenerate shoot progenitors and completion of regeneration.

11. Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ: **LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development [Internet]**. *Proc Natl Acad Sci U S A* 2001, **98**:11806-11811.

12. • Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, Inoue Y, Seki M, Sakakibara H, Sugimoto K *et al.*: **The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in Arabidopsis [Internet]**. *Curr Biol* 2011, **21**:508-514.
This study demonstrates that expression of a wounding induced gene *WIND1* is sufficient to induce somatic cell dedifferentiation to form pluripotent callus in a hormone independent manner.

13. Gordon SP, Heisler MG, Reddy GV, Ohno C, Das P, Meyerowitz EM: **Pattern formation during de novo assembly of the Arabidopsis shoot meristem**. *Development* 2007, **134**:3539-3548.

14. Gallois J-L, Nora FR, Mizukami Y, Sablowski R: **WUSCHEL induces shoot stem cell activity and developmental plasticity in the root meristem [Internet]**. *Genes Dev* 2004, **18**:375-380.

15. Kral N, Hanna Ougolnikova A, Sena G: **Externally imposed electric field enhances plant root tip regeneration**. *Regeneration (Oxf)* 2016, **3**:156-167.

16. Atta R, Laurens L, Boucheron-Dubuisson E, Guivarc'h A, Carnero E, Giraudat-Pautot V, Rech P, Chriqui D: **Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown in vitro [Internet]**. *Plant J* 2009, **57**:626-644.

17. Che P, Lall S, Howell SH: **Developmental steps in acquiring competence for shoot development in Arabidopsis tissue culture**. *Planta* 2007, **226**:1183-1194.

18. Sugimoto K, Meyerowitz EM: **Regeneration in Arabidopsis tissue culture [Internet]**. In *Plant organogenesis: methods and protocols.* Edited by De Smet I. Humana Press; 2013:265-275.

19. Gordon SP, Heisler MG, Reddy GV, Ohno C, Das P, Meyerowitz EM: **Pattern formation during de novo assembly of the Arabidopsis shoot meristem [Internet]**. *Development* 2007, **134**:3539-3548.

20. Zhang T-Q, Lian H, Tang H, Dolezal K, Zhou C-M, Yu S, Chen J-H, Chen Q, Liu H, Ljung K *et al.*: **An intrinsic microRNA timer**

regulates progressive decline in shoot regenerative capacity in plants [Internet]. *Plant Cell Online* 2015, **27**:349-360.

21. Akama K, Shiraishi H, Ohta S, Nakamura K, Okada K, Shimura Y: **Efficient transformation of *Arabidopsis thaliana*: comparison of the efficiencies with various organs, plant ecotypes and Agrobacterium strains [Internet]**. *Plant Cell Rep* 1992, **12**:7-11.

22. Kareem A, Radhakrishnan D, Wang X, Bagavathiappan S, Trivedi ZB, Sugimoto K, Xu J, Mähönen AP, Prasad K: **Protocol: a method to study the direct reprogramming of lateral root primordia to fertile shoots**. *Plant Methods* 2016, **12**.

23. •• Rosspopoff O, Chelysheva L, Saffar J, Lecorgne L, Gey D, Caillieux E, Colot V, Roudier F, Hilson P, Berthome R *et al.*: **Direct conversion of root primordium into shoot meristem relies on timing of stem cell niche development [Internet]**. *Development* 2017, **144**:1187-1200.

The author has performed a detailed dissection of the cellular and molecular events taking place during direct *de novo* shoot regeneration. This work uncovers the necessity for the presence of an apical stem cell niche in a competent LRP for successful shoot regeneration.

24. Chatfield SP, Capron R, Severino A, Penttila PA, Alfred S, Nahal H, Provart NJ: **Incipient stem cell niche conversion in tissue culture: using a systems approach to probe early events in WUSCHEL-dependent conversion of lateral root primordia into shoot meristems [Internet]**. *Plant J* 2013, **73**:798-813.

25. •• Sugimoto K, Jiao Y, Meyerowitz EM: **Arabidopsis regeneration from multiple tissues occurs via a root development pathway [Internet]**. *Dev Cell* 2010, **18**:463-471.

The authors provide definitive proof of the necessity of initial root developmental pathway during competent callus formation. This changed the pre-existing notion that callus is a dedifferentiated tissue.

26. Lavenus J, Goh T, Roberts I, Guyomarc'h S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennett M, Laplaze L: **Lateral root development in Arabidopsis: fifty shades of auxin**. *Trends Plant Sci* 2013, **18**:450-458.

27. Ikeuchi M, Sugimoto K, Iwase A: **Plant callus: mechanisms of induction and repression [Internet]**. *Plant Cell* 2013, **25**:3159-3173.

28. Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, Xu L: **WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in Arabidopsis**. *Plant Cell* 2014, **26**:1081-1093.

29. Sugimoto K, Gordon SP, Meyerowitz EM: **Regeneration in plants and animals: dedifferentiation, transdifferentiation, or just differentiation? [Internet]**. *Trends Cell Biol* 2011, **21**:212-218.

30. Laplaze L, Parizot B, Baker A, Ricaud L, Martiniere A, Auguy F, Franche C, Nussaume L, Bogusz D, Haseloff J: **GAL4-GFP enhancer trap lines for genetic manipulation of lateral root development in *Arabidopsis thaliana***. *J Exp Bot* 2005, **56**:2433-2442.

31. • Shang B, Xu C, Zhang X, Cao H, Xin W, Hu Y: **Very-long-chain fatty acids restrict regeneration capacity by confining pericycle competence for callus formation in Arabidopsis [Internet]**. *Proc Natl Acad Sci U S A* 2016, **113**:5101-5106.

The author identifies very long chain fatty acids as upstream molecules which control the transcription of the *ALF4* gene that is important in callus formation as well as lateral root formation.

32. Fukaki H, Tameda S, Masuda H, Tasaka M: **Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis**. *Plant J* 2002, **29**:153-168.

33. Celenza JL, Grisafi PL, Fink GR: **A pathway for lateral root formation in *Arabidopsis thaliana* [Internet]**. *Genes Dev* 1995, **9**:2131-2142.

34. Fan M, Xu C, Xu K, Hu Y: **LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in Arabidopsis regeneration [Internet]**. *Cell Res* 2012, **22**:1169-1180.

35. Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M: **ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis**. *Plant Cell* 2007, **19**:118-130.

36. Lee HW, Kim NY, Lee DJ, Kim J: **LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in Arabidopsis**. *Plant Physiol* 2009, **151**:1377-1389.

37. Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B: **The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche**. *Cell* 2004, **119**:109-120.

38. Prasad K, Grigg SP, Barkoulas M, Yadav RK, Sanchez-Perez GF, Pinon V, Blilou I, Hofhuis H, Dhonukshe P, Galinha C *et al.*: **Arabidopsis PLETHORA transcription factors control phyllotaxis**. *Curr Biol* 2011, **21**:1123-1128.

39. Hofhuis H, Laskowski M, Du Y, Prasad K, Grigg S, Pinon V, Scheres B: **Phyllotaxis and rhizotaxis in Arabidopsis are modified by three PLETHORA transcription factors**. *Curr Biol* 2013, **23**:956-962.

40. • Iwase A, Harashima H, Ikeuchi M, Rymen B, Ohnuma M, Komaki S, Morohashi K, Kurata T, Nakata M, Ohme-Takagi M *et al.*: **WIND1 promotes shoot regeneration through transcriptional activation of ENHANCER OF SHOOT REGENERATION1 in Arabidopsis [Internet]**. *Plant Cell Online* 2016 http://dx.doi.org/10.1105/tpc.16.00623.

The authors have demonstrated that a wound induced transcription factor WIND1 regulates callus formation and shoot regeneration by binding to the *ESR1* promoter and activating its expression. The findings contribute to our understanding of the molecular pathway connecting wound signaling and shoot regeneration.

41. Stetler DA, Laetsch WM: **Kinetin-induced chloroplast maturation in cultures of tobacco tissue [Internet]**. *Science* 1965, **149**:1387-1388.

42. • Motte H, Vercauteren A, Depuydt S, Landschoot S, Geelen D, Werbrouck S, Goormachtig S, Vuylsteke M, Vereecke D: **Combining linkage and association mapping identifies RECEPTOR-LIKE PROTEIN KINASE1 as an essential Arabidopsis shoot regeneration gene**. *Proc Natl Acad Sci U S A* 2014, **111**:8305-8310.

A comprehensive study of several accessions of *Arabidopsis* using association and linkage analysis which helps in uncoupling several intermediate stages of *de novo* shoot regeneration.

43. Landrein B, Kiss A, Sassi M, Chauvet A, Das P, Cortizo M, Laufs P, Takeda S, Aida M, Traas J *et al.*: **Mechanical stress contributes to the expression of the STM homeobox gene in Arabidopsis shoot meristems**. *Elife* 2015, **4**:e07811.

44. Shemer O, Landau U, Candela H, Zemach A, Eshed Williams L: **Competency for shoot regeneration from Arabidopsis root explants is regulated by DNA methylation [Internet]**. *Plant Sci* 2015, **238**:251-261.

45. Zhang TQ, Lian H, Zhou CM, Xu L, Jiao Y, Wang JW: **A two-step model for de novo activation of WUSCHEL during plant shoot regeneration [Internet]**. *Plant Cell* 2017 http://dx.doi.org/10.1105/tpc.16.00863.

46. Li W, Liu H, Cheng ZJ, Su YH, Han HN, Zhang Y, Zhang XS: **DNA methylation and histone modifications regulate de novo shoot regeneration in Arabidopsis by modulating WUSCHEL expression and auxin signaling [Internet]**. *PLOS Genet* 2011, **7**:1-12.

47. Mayer KF, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T: **Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem**. *Cell* 1998, **95**:805-815.

48. Schoof H, Lenhard M, Haecker A, Mayer KFX, Jürgens G, Laux T: **The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes [Internet]**. *Cell* 2000, **100**:635-644.

49. Liu Z, Li J, Wang L, Li Q, Lu Q, Yu Y, Li S, Bai M-Y, Hu Y, Xiang F: **Repression of callus initiation by the miRNA-directed interaction of auxin-cytokinin in *Arabidopsis thaliana***. *Plant J* 2016, **87**:391-402.

50. •• Efroni I, Mello A, Nawy T, Ip P-L, Rahni R, DelRose N, Powers A, Satija R, Birnbaum KD: **Root regeneration triggers an embryo-like sequence guided by hormonal interactions**. *Cell* 2016, **165**:1721-1733.

Using a variety of techniques, the authors reveal that upon damage, the root regeneration program recapitulates the developmental stages of embryonic patterning. They also demonstrate that patterning is guided by hormonal signaling domains.

51. Efroni I, Ip P-L, Nawy T, Mello A, Birnbaum KD: **Quantification of cell identity from single-cell gene expression profiles**. *Genome Biol* 2015, **16**:9.

52. Campilho A, Garcia B, Toorn HVD, Wijk HV, Campilho A, Scheres B: **Time-lapse analysis of stem-cell divisions in the *Arabidopsis thaliana* root meristem**. *Plant J* 2006, **48**:619-627.

53. Calder G, Hindle C, Chan J, Shaw P: **An optical imaging chamber for viewing living plant cells and tissues at high resolution for extended periods [Internet]**. *Plant Methods* 2015, **11**:22.

54. Heisler MG, Ohno C, Das P, Sieber P, Reddy GV, Long JA, Meyerowitz EM: **Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem**. *Curr Biol* 2005, **15**:1899-1911.

55. Reddy GV, Heisler MG, Ehrhardt DW, Meyerowitz EM: **Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex of *Arabidopsis thaliana***. *Development* 2004, **131**:4225-4237.

56. Hu B, Zhang G, Liu W, Shi J, Wang H, Qi M, Li J, Qin P, Ruan Y, Huang H *et al.*: **Divergent regeneration-competent cells adopt a common mechanism for callus initiation in angiosperms [Internet]**. *Regeneration* 2017 http://dx.doi.org/10.1002/reg2.82. [accepted online].