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Regrowing the damaged or lost body parts

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Plants display extraordinary ability to revive tissues and organs lost or damaged in injury. This is evident from the root tip restoration and classical experiments in stem demonstrating re-establishment of vascular continuity. While recent studies have begun to unravel the mechanistic understanding of tissue restoration in response to injury in underground plant organs, the molecular mechanisms of the same in aerial organs remain to be ventured deeper. Here, we discuss the possibility of unearthing the regulatory mechanism that can confer universal regeneration potential to plant body and further provide a comprehensive understanding of how tissue and organ regeneration gets triggered in response to mechanical injury and later gets terminated after re-patterning and regaining the appropriate size.

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Introduction

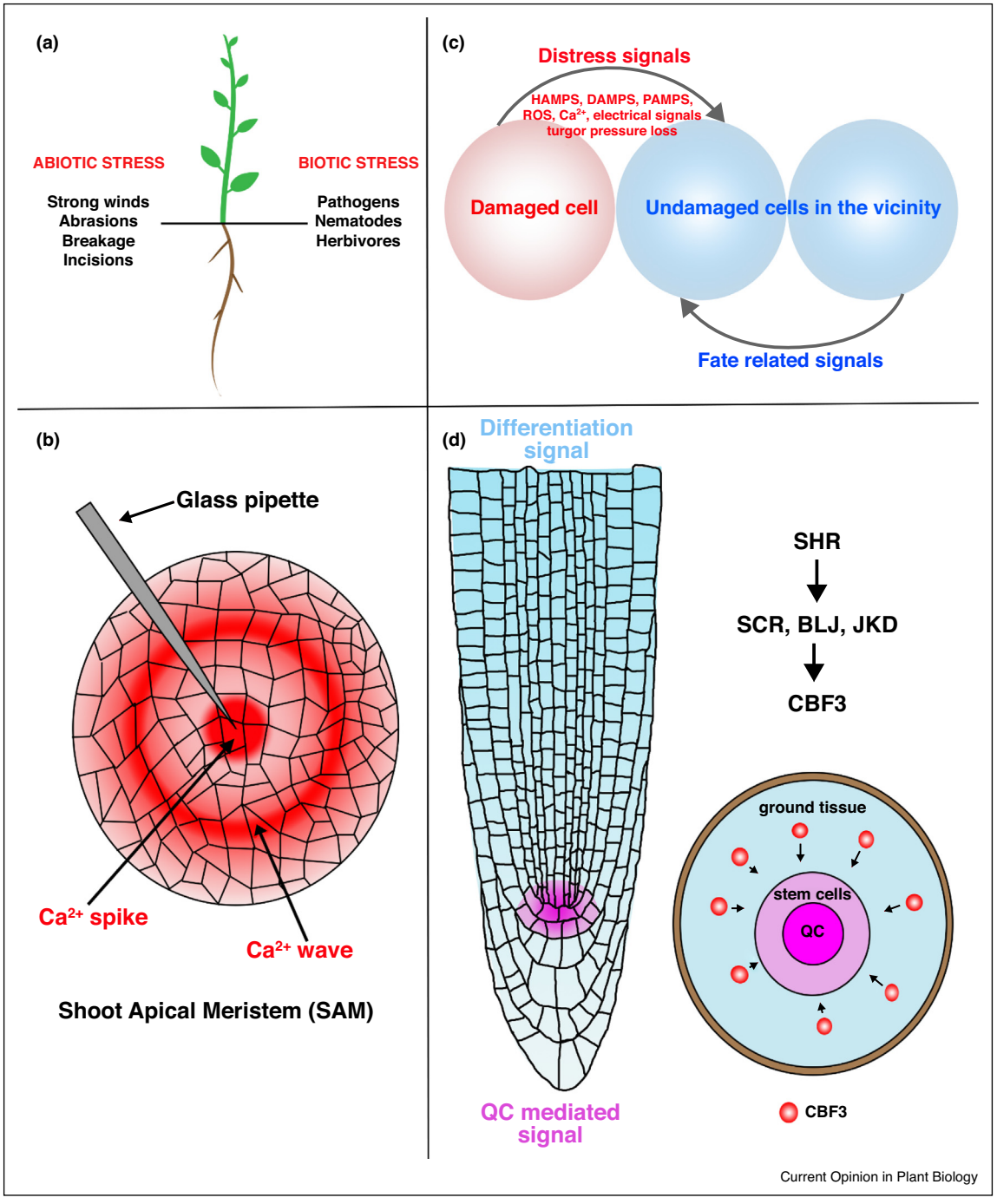
Plants, despite being sessile exhibit exceptional ability to perceive and respond to environmental stimuli. They use a variety of mechano-sensing, electrical and chemical signalling to respond to external stimuli as evident from some of their responses such as drooping of Mimosa leaves and closing of Venus flytrap [1,2]. The detection and prompt response to external stimuli displayed by plants are beneficial during their growth as they encounter severe environmental stress by either biotic factors such as pathogens, nematodes, and herbivores or abiotic factors such as strong winds, abrasions, breakage, and incisions (Figure 1a). Plants efficiently respond to injury and often restore the lost organ. Unlike animals, plants cannot recruit specialised cells dedicated to wound healing as cell migration is absent. Instead, cells in the vicinity of the wound show remarkable plasticity and get reprogrammed to meet the urgent demand of repair, essential for their survival as unattended open wounds can lead to infections and eventually death [3]. So how do plants mount a timely response and restore the damaged tissues? They use a combination of electrical [4], chemical [5], mechanical [1] and positional cues [6,7]. Regeneration at the wounded region occurs either by complete organ restoration or by replacing few cells by cell division and differentiation [8,9]. However, the mechanism underlying wound perception, repair and repatterning in the context of a growing plant is yet to be fully explored. This review describes the sequential events that occur in response to local damages in growing plants.

Wound perception and signalling

Wounding is the primary trigger for regeneration in plants and animals. But how do they detect the wound? In animals, stretched skin is disrupted by a wound causing the skin to retract. This mechanical alteration of the environment contributes to mechano-tactic guiding of specialised cells to the wound site and orchestrate their differentiation eventually culminating in wound healing [10]. However, plants adopt different mechanisms as cell migration is absent owing to their rigid cell walls.

Wounding alters the cell wall integrity (CWI) of the cells in the vicinity. The perception of this altered CWI by stretch activated mechano-sensitive channels on the plasma membrane is a key event during wound detection [11,12]. Since the damaged cells loses turgor pressure, cells adjoining the damaged cells experience a sudden imbalance in stress and loss of initial radial alignment of microtubules thereby changing their mechanical properties [13]. In addition, DAMPs (Damage Associated Molecular Patterning) such as extracellular ATP (eATP), oligogalacturonic fragments (OG), glutamate and sucrose, Pathogen derived PAMPs (Pathogen Associated Molecular Patterning) and herbivore derived HAMPs (Herbivore Associated Molecular Patterning) are released from damaged cells [14–16]. Thus, the altered CWI, altered mechanical properties of adjacent cells, a sudden increase in the extracellular concentration of sucrose, DAMPs, PAMPs and HAMPs indicate tissue disruption in the vicinity. These probably act as the distress signals emanating from the damaged cell, which upon perception by neighbouring cells trigger a secondary signal directing wound response. A local injury often elicits two kinds of responses: systemic immune response and healing response. eATPs and OGs trigger Ca^{2+} influx and ROS

Figure 1



Schematic illustration of tissue injuries and signalling in plant.
(a) Various biotic and abiotic factors inflict damage to aerial and underground plant parts during normal growth.
(b) Local ablation of shoot apical meristem produces two kinds of calcium signals- i) Ca^{2+} spike: cells in the immediate vicinity of the wound exhibit a surge in cytosolic Ca^{2+} ii) Ca^{2+} waves that are radiated away from damaged cells to undamaged cells [21•].
(c) Distress signals are released from damaged cells to the adjoining undamaged cells. These cells could also perceive fate related signals from other neighbouring undamaged cells.
(d) A growing root exhibit two kinds of positional signals. Differentiation signals released from mature cells of root promote cell differentiation while QC-mediated signals inhibit differentiation promoting signals [31].
Cross section of root showing ground tissue and stem cell niche where, transcription factor CBF3 moves out of ground tissue to maintain stem cell niche and to confer regeneration [32••]. CBF3 is regulated by SCR, BLJ(BLUEJAY) and JKD(JACKDAW) which in turn are direct targets of SHR [32••,69].

production respectively, indicating their perception as an early event in plant wound signaling [15]. Perception of glutamate by ion channels of Glutamate Receptor-Like (GLR) protein elicit defence signal propagation by altering the cytosolic Ca^{2+} concentration, wherein defence signals are propagated as electrical signals. Cytosolic Ca^{2+}concentration, ROS, electrical signals contribute to the trio signalling that support both local and systemic immune response [16,17]. PAMPs and HAMPs stimulate wound induced synthesis of jasmonic acid (JA) [18] that translocates from damaged to undamaged region where, the perception and subsequent JA signalling activates defence response [19]. Thus, DAMPs, PAMPs, HAMPs, calcium, ROS and JA are involved in signalling networks that initiate systemic immune responses upon wounding.

In addition to immune responses, wound healing and regeneration are also elicited upon local injury. Calcium on entry into cell acts as a master regulator of wound healing. A study by wounding epithelial tissue of Drosophila pupae via pulsed laser ablation, report two sequential waves of calcium spreading into the neighbouring cells [20]. Occurrence of similar pattern of calcium dynamics in shoot apical meristem of *Arabidopsis* where local cell ablation resulted in a calcium spike at the immediate vicinity and calcium wave propagating away from the site of injury, suggests the possibility of convergence of signalling from different damage mechanisms such as single-cell damage and tissue damage, on increasing cytosolic Ca^{2+} concentration to regulate wound healing (Figure 1b) [21•]. It is likely that the sustained calcium spike in the immediate vicinity of the wound contributes to proliferation responses while the propagating calcium wave contributes to the immune responses.

Thus in plants, a rapid influx of calcium into the cell via stretch activated mechano-sensitive ion channels, as well as the DAMPs, PAMPs and HAMPS could probably stimulate a downstream signalling cascade which can alter the molecular and hormonal environment in the cells adjacent to the wound. Such hormonal and molecular alterations instrumental in reprogramming the cells in response to wounding will be discussed in the subsequent sections.

Gene activation and cellular reprogramming

Cells adjoining the wound probably experience an abrupt loss of communication from cells that lie on the side of the wound. The communication loss from one side could cause the genes to disengage from the ongoing developmental regulatory network and become readily available to respond to distress signals emanating from the nearby damaged cell.

The distress signals are perceived by the cells in the vicinity of a wound within seconds of injury. However, it takes hours to initiate regeneration responses. Understanding the delay between the perception and regeneration initiation remains fragmentary. One of the prime focuses of most of the recent studies is exploring the activation of a variety of genes and hormonal upregulation in response to wounding. However, the temporal order of hormonal surge and gene activation where one can be causal for the other needs to be resolved. A rise in activation of various genes including stem cell regulators accompanied by hormonal surge was reported in several experiments using cell ablation and excision studies in plants [22,23]. Molecular mechanisms that bring about an increase in gene activation and hormones can be partly attributed to epigenetic modifications in their loci. For example, an elegant study which demonstrates root regeneration from shoot, reports the key role of epigenetic regulators in facilitating the activation of a hub of genes upon injury where, wound induced transient surge in JA upregulates auxin biosynthesis gene via histone methylation [24].

Laser ablation of root meristem cells indicates that a surge in auxin precedes the rise in gene activation. Laser ablation of quiescent centre (QC) cells shifts the auxin response shootward during early hours post ablation and contributes to cell fate changes in adjoining cells via expression of root stem cell regulators *PLETHORA (PLT), SHORTROOT(SHR) and SCARECROW(SCR)* [22]. Recent studies report the upregulation of stem cell regulators upon targeted ablation of cells of root meristem several hours post ablation [25•] suggesting that the initial rise in auxin promotes the build-up of root stem cell regulators after injury. However, whether surge in auxin responses re-activate the cell fate determinants is yet to be established.

Root tip excision studies report an increase in auxin levels accompanied by activation of stem cell regulators in the neighbouring cells, but the temporal order of auxin surge and activation of stem cell regulators remain unknown [23]. Recent studies report a rapid accumulation of PLT2 near the cut site within few hours, before any significant change in auxin response, while a higher auxin response by virtue of local auxin biosynthesis occurs relatively later, that drives root tip regeneration [26••,27•]. Corroborating with the fact that PLT2 activates local auxin biosynthesis gene *YUCCA3(YUC3)* in the context of root development, it is likely that rapid burst of PLT2 builds up necessary auxin responses via local auxin biosynthesis in response to root tip excision [28].

Very much in line with the role of PLT2 in root tip regeneration, other *PLTs(PLT3,5,7)* in shoot activates local auxin biosynthesis in a coherent feed-forward loop with *CUP SHAPED COTYLEDON 2(CUC2)* thereby, contributing towards generating effective auxin response in wounded aerial organs [29,30•]. Apart from a few studies, it remains unresolved whether the upregulation

of hormones drives the surge in cell fate determinants or *vice versa* in other contexts of wound healing. Given ample evidence, it is highly unlikely that the interplay between hormones and cell fate determinants follow a linear relation. The mounting evidence during normal development suggests a regulatory feedback loop between the stem cell determinants and hormone responses in both aerial and underground organs. Presumably, several such regulatory loops operate between the cell fate determinants and hormones upon injury, resulting in wound healing and organ regeneration.

Wound healing and tissue or organ restoration

Re-activation of a variety of genes in undamaged cells in the vicinity of the wound leads to regeneration. Depending on the nature of injury and the context of damage, the regeneration responses are either confined to healing in the form of local cell proliferation or organ restoration. During normal plant development, the cell-to-cell communication in growing organ directs its growth and patterning. For example, in root it is proposed that QC signal inhibits differentiation of contacting cells, while positional signal for proper differentiation is conferred to them by more mature cells (Figure 1d) [31]. Such a signalling mechanism also operates in replacing damaged cells at the root tip [6,31]. Corroborating with this notion it can be proposed that distress signals from damaged cells reactivate the stem cell regulators in the contacting cells to trigger their proliferation and maintain them in undifferentiated state whilst, neighbouring undamaged cells confer them signals for differentiation (Figure 1c).

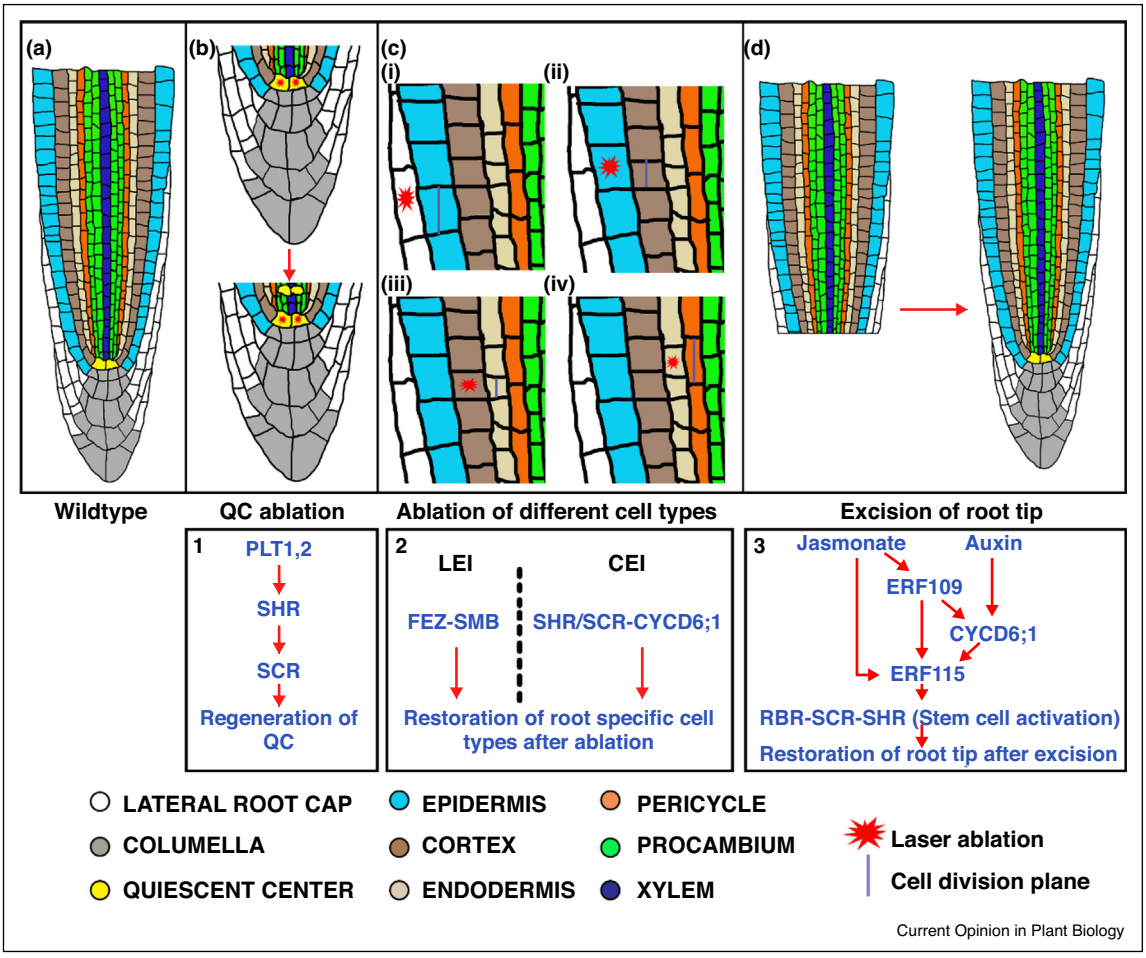
The displacement of QC towards the proximal part of root meristem upon QC ablation demonstrates the re-activation of root stem cell regulators (Figure 2b) [22]. In addition to QC, other injured cells of the root meristem can be replaced by positional cues emanating from the undamaged neighbouring cells [6,31]. Very much in line with these findings cell division by cells of inner cell files replaces ablated cells of outer cell files. This restorative cell division was highly compromised in mutants defective in stem cell maintenance (Figure 2c) [25•]. A question arises whether injury induced genes act autonomously or can act non-cell autonomously as well during regeneration. Interestingly, a recent study shows that signals originate not only from QC, but also from ground tissue wherein BIRD family genes regulate C-REPEAT BINDING FACTOR 3 (CBF3) which move out of the ground tissue and confer regeneration to stem cell niche (Figure 1d) [32••]. Thus it is quite conceivable that pre-existing endogenous cue responds to injuries and drives the regeneration process.

What happens when an organ is lost in injury? Removal of root tip results in loss of stem cell niche. Interestingly, plants are capable of regenerating their missing root tip, suggesting fully functional stem cell niche is not required for root tip regeneration [23]. Though, the root meristem harbours dividing cells, only a portion of it regenerate upon wounding, as the efficiency of root tip regeneration sharply decreases toward the proximal part of the meristem [23,26••]. The non-uniform distribution of regeneration ability along the organ axis is not confined to plants but also other kingdoms, as zebrafish fin regeneration displays a similar pattern (Figure 3a) [33]. This raises an interesting question, what imposes the boundary on organ regeneration potential?

Recent discovery unravelled the existence of a regeneration competence zone attributed to a gradient expressed transcription factor PLT2 in the root meristem, whose autoactivation guides regeneration (Figure 3b). Relatively higher expression of PLT2 in the competence zone (distal end of meristem) contributes to high regeneration potential while, low level of PLT2 in the non-competence zone (proximal end of root meristem) impedes the regeneration ability. When the high and low expression domains of PLT2 are reversed, the regeneration potential also reverses accordingly without altering the meristem size. In multiple mutant combination of redundant *plt*, transient downregulation of endogenous PLT2 in the competence zone leads to cessation of regeneration. These findings explain why *plt1*, *plt2* mutants still exhibit regeneration where redundant PLTs can substitute this function [23]. In wild type, transient overexpression of PLT2 in differentiating cells of non-competence zone confers the regeneration potential by upregulation of endogenous PLT2 expression. However, sustained PLT2 overexpression beyond a threshold that can increase the meristem length fails to restore the root tip after excision, leaving only residual cell proliferation at the cut end. The findings demonstrate the dosage-dependent role of gradient expressed transcription factor in root tip regeneration, and decouple regeneration potential of an organ from its size as well as local cell proliferation response from complete organ restoration (Figure 3b) [26••]. In addition to the internal cues, organ regeneration efficiency can be manipulated by external cues, as the proximal end of meristem that regenerates poorly exhibits efficient regeneration in response to weak electrical pulse and external auxin [27•,34].

The wealth of information on regeneration obtained from laboratory studies that mimic field conditions provide a better understanding of the molecular mechanisms dictating regeneration under natural growing conditions. Owing to the injury to roots imparted by nematodes, perennial woody plants evolve a greater adaptation as they remain rooted for a longer period than annual plants [35]. Single cell ablation studies mimicking cyst nematode-mediated cell damage, reveals the protective effect of ethylene response genes against nematodes [36••]. A recent study using both QC ablation and root tip excision as a model shows a rapid increase in JA and auxin which

Figure 2

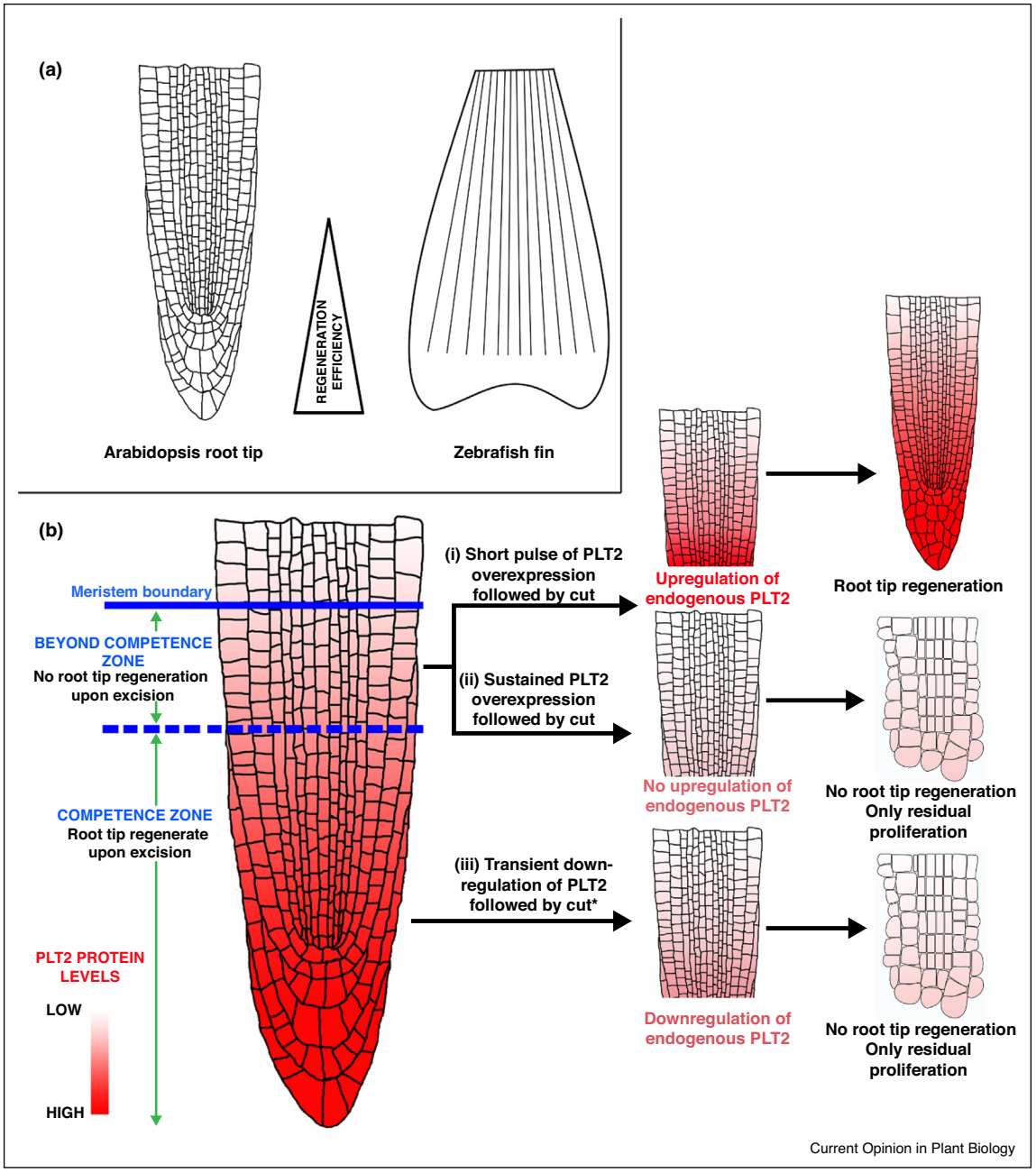


Schematic diagram representing regeneration of specific cell types and tissues in root tip.
(a) Wildtype root showing different cell files.
(b) Root cell fate determinants (PLT, SCR and SHR) helps in the respecification of the laser ablated QC a few cell layers above the original site [22].
Box 1: The flow diagram represents the temporal order of activation of root cell fate determinants PLT1, PLT2, SHR and SCR for QC regeneration.
(c) Restorative cell division replacing the ablated (i) Lateral root cap cell (ii) epidermal (iii) cortical (iv) endodermal cell of root meristem [6,25•].
Box 2: Lateral Root Cap/Endodermis Initial (LEI) derived cells follow FEZ-SMB (SOMBRERO) pathway while, Cortex-Endodermis Initial (CEI) derived cells follow SHR/SCR-CYCD6;1 pathway to activate the restoration of ablated cells [25•].
(d) In response to damage, JA and auxin activates a network of proteins leading to stem cell activation and restoration of root tip.
Box 3: Convergence of JA and auxin signalling leads to stem cell activation (RBR-SCR-SHR protein network) via ERF115, imperative for root tip restoration after excision [39••].

result in activation of stem cell regulator RBR-SCR-SHR (RETINOBLASTOMA-RELATED-SCARECROW-SHORTROOT) through *ERF109 (ETHYLENE RESPONSE FACTOR 109)*, *ERF115* and *CYCD6;1 (CYCLIND6;1)* enabling restoration of root tip (Figure 2d). *ERF115* transcriptionally regulate *WOUND INDUCED DEDIFFERENTIATION (WIND1)* to promote root tip regeneration [37,38]. In addition to root tip regeneration and growth after nematode invasion, JA pathway also promotes the reproductive success of a nematode [39••]. Considering the mutually beneficial nature of this JA signalling, it can be presumed that the mechanism co-evolved in both plant and nematode.

We know that in laboratory conditions, electrical pulse and cell fate determinants influence root tip regeneration [34,39••]. However the relation between the two is not yet established. Interpreting this relation can help in comprehending the molecular mechanisms driving root tip regeneration during nematode infection, as soil dwelling fauna can impair the intrinsic electrical signalling in root [40,41].

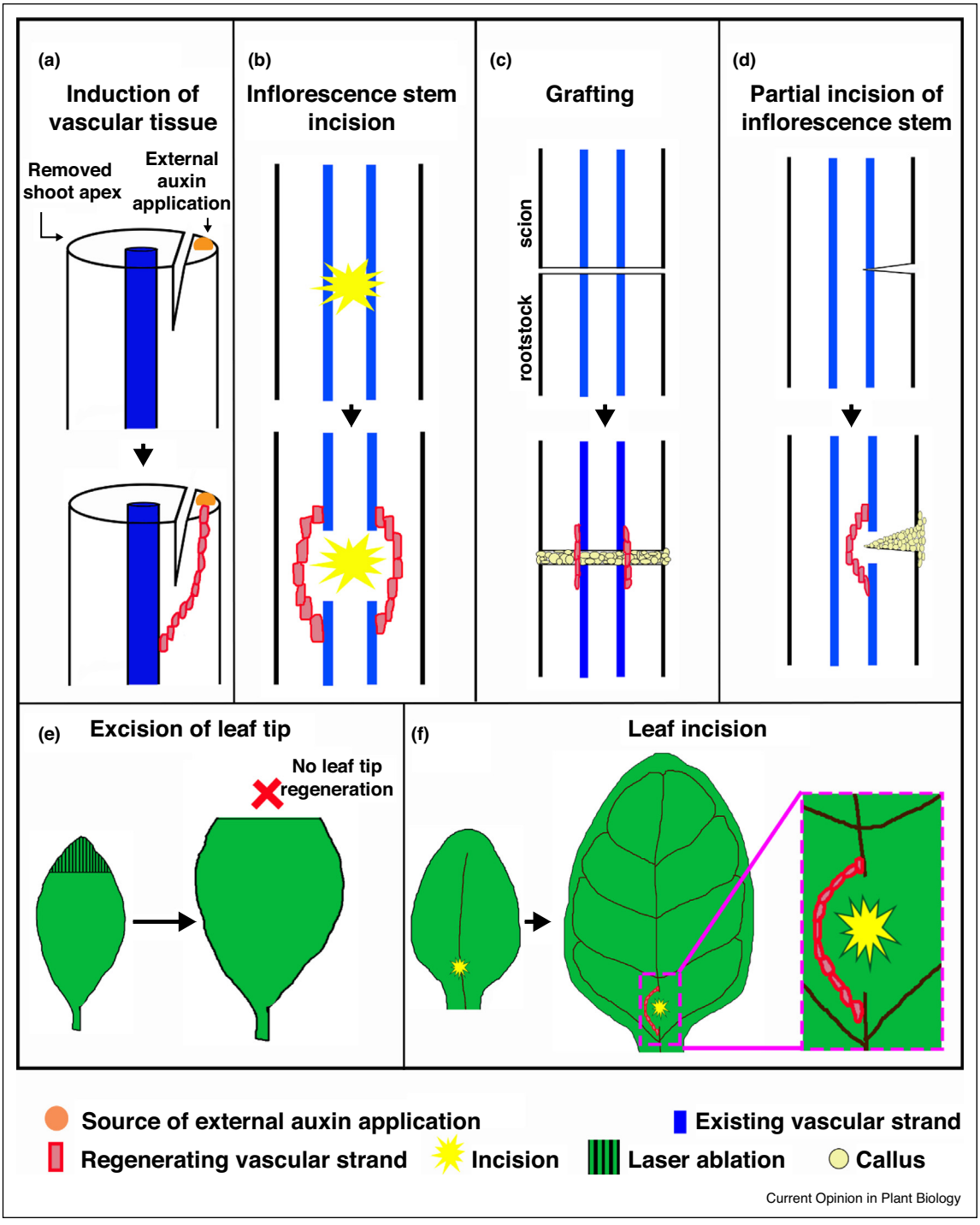
Figure 3



Various degree of regeneration potential along the organ axis.
(a) Regeneration efficiency is non-uniform along the proximo-distal axis in *Arabidopsis* root tip and Zebrafish fin [23,26••,33].
(b) Schematic diagram represent gradient expression of PLT2 transcription factor in root [70] and the effect of modulation of PLT2 expression level on root tip regeneration.
*Transient downregulation of PLT2 in the background of multiple *plt* mutant combination [26••].

Elucidation of comprehensive mechanisms by which the underground plant organ respond to injuries makes one wonder if similar mechanisms operate in aerial organs as well. Local cell proliferation in the form of callus occurs upon partial incision [29,42], girdling [43–45], grafting [46], and abrasion in stem [30•] (Figure 4) (Regeneration responses during grafting have been reviewed in detail in many other excellent reviews. Therefore we will not be discussing here in the context of grafting [47–51]). Callus formed in response to wounding is a composite tissue arising from multiple cell types including cortex, pith and vascular cell, but identification of its exact origin awaits

Figure 4



Schematic diagram displaying wound repair and vascular regeneration in growing stem and leaf.
(a) New vascular strand forms from site of external auxin application, on a flap of epicotyl tissue which is separated from main vascular strand by a deep cut. The new vascular strand grows to unite with the existing vascular strand [54].
(b) In response to stem injury that disconnect vascular strands, newly regenerating vascular tissue around a damaged site reconnects to parental strand [57,64].
(c) Callus formation between stock and scion is followed by vascular reunion during grafting [50].
(d) Regenerating vascular tissues circumvent the site of partial incision in inflorescence stem and reconnects parental strand [30•].
(e) Leaf tip fails to regenerate upon laser assisted excision [66].
(f) Regenerating vascular strands form a D-loop to re-instate the vascular continuity in mid-rib, which was disrupted upon incision [30•].

cell lineage tracing [29]. Callus cells exhibit versatile nature, by virtue of which they can switch from one lineage to another for functional restoration. This is validated by the restoration of bark tissue from axial parenchyma derived callus in response to girdling [43].

Callus acts as an adhesion material to seal the wound and to provide immediate protection. In deeper wounds such as inflorescence stem incision or grafting where the vasculature has been cut off, callus formation is accompanied by re-establishment of vascular continuity. However, enhanced callus formation occurs when vascular continuity fails to re-establish, which compromises tissue regeneration. This draws attention to the inverse proportionality of callus formation to tissue restoration [30•,50,52]. Therefore, tissue restoration is determined by size of the wound, wherein the inability to regain vascular continuity in case of extensive damage can be attributed to the failure to establish optimum auxin flux [53], as inferred from the canalization hypothesis proposed by Tsvi Sachs [54,55].

Simple and elegant experiments by Tsvi Sachs in various plant species, showed that vascular strands could be induced from mature parenchymatous tissues when the auxin flows from a source to sink (Figure 4a). The growing leaves or site of external auxin application is the source while, the sink is the end of the wounded tissue with a relatively lower level of auxin concentration. Regeneration requires the successful re-establishment of polar auxin transport from source to sink [52,54–57]. In comparison to dicots, monocots display relatively poor vascular regeneration efficiency [58,59] due to the absence of vascular cambium [57,60]. This is evident from the weak regeneration in the form of discontinuous strand formation in Zea mays. Moreover, injury in older internodes completely fails to regenerate [61]. The necessity of vascular continuity re-establishment during plant organ regeneration is analogous to the indispensable requirement of nerves in salamander limb regeneration [62]. Thus re-establishment of tissue continuity is instrumental for functional restoration of the organ.

How does the tissue on either side of the wound recognise each other? Upon partial incision of *Arabidopsis* inflorescence stem, auxin transport is disrupted and the unequal distribution of auxin result in differential expression of many transcription factors on either side of the wound [29]. Such an asymmetrically localised expression of transcription factors such as *RAP2.6L(RELATED TO APETALA2.6L)* and *ANAC071* (*Arabidopsis* NAC domain containing protein 71) around the wound site is essential for tissue recognition to direct vascular reunion [29,63].

A local injury interrupting vascular connection in epicotyls and inflorescence stem can be repaired, wherein the regenerating vascular strands bypass the wounded area to connect to pre-existing strands [42,64]. Only recently, lateral organs such as leaves were explored for re-establishment of mid rib continuity as a D-shaped loop where, the distance between the regenerating strand and the parent strand dictates the length/size of the vascular strand that forms the D-loop (Figure 4f) [30•]. However, what guides the vascular strands through new venation path remains unknown. In light of these observations where vascular strands regenerate around the wound, it is tempting to speculate that combinatorial contacts guide the orientation of the cell division plane in the newly re-specified vascular cells either by imposing appropriate mechanical forces or by providing biochemical cues [65]. However, *Arabidopsis* leaf tip when excised, is not replaced (Figure 4e) [66] suggesting that only physiologically relevant tissues within growing leaves are regenerated.

Studies on the molecular mechanisms determining the path of vascular tissue regeneration in aerial parts are fragmentary. At least in leaves a coherent feed forward loop, where PLT3,5,7 and CUC2 both upregulate auxin biosynthesis gene *YUCCA4(YUC4)* to meet the necessary auxin surge at the damaged end, drive vascular regeneration and reunion to its parental strands [30•].

Though genes for regeneration in root and shoot are different, auxin surge in response to injury acts as a common regulatory module to drive regeneration in both cases. It seems auxin driven tissue polarity, independent of auxin transporters, acts upstream of polar auxin transport and signalling to guide the path of vascular formation in leaves. It is likely that such an auxin-dependant tissue polarity mechanism also operates during vascular regeneration in leaf [67•].

An organ is said to be restored only when it attains the appropriate shape and size pertaining to that particular developmental stage of the plant. This is evident from restoration of root tip and re-establishment of vascular continuity. Thus it is plausible that the mechanism of organ restoration is tightly coupled with cellular differentiation and it is likely to follow normal developmental program.

Perspective

Recent studies have begun to uncover a number of transcription factors induced in response to injury, whose upregulated expression control wound repair. Several such studies draw a link between activity of wound-induced transcription factors and surge in hormone signalling pathways. Incorporation of such studies while elucidating the injury-mediated gene regulatory network provides insight into innate regeneration mechanisms in plants. This will be beneficial for a deeper understanding of the interplay between the mechanism of regeneration and the normal developmental process of the tissue or organ. Such a comparison is also likely to unravel

regulatory modules that can distinguish the ability of tissue repair from its formation and this can be used to engineer organ restoration without interfering with its normal developmental program.

The mechanism by which undamaged cells in the vicinity of the wound detect the injury and relay the signals to elicit the molecular and cellular events require further investigation (Figure 1c). Chemical and electrical signals emanating from damaged cells can serve as possible messengers. In addition to these signals, changes in the mechanical properties of adjoining undamaged cells can contribute towards molecular and cellular repatterning in response to injury [13,68].

One of the quick cellular responses to injury is cell proliferation in the direction of wound. This polarised proliferation response must be tightly controlled to generate optimum pool of cells for subsequent cell-fate transitions and repatterning. A very fine balance between the cellular processes such as cell division and cell-fate transitions during tissue or organ regeneration is likely to be well-orchestrated with a number of regulatory feedback loops and entangled regulatory interactions. Unravelling these regulatory interactions will be instrumental to a deep mechanistic understanding of successful regeneration of the tissue or organ of appropriate size.

The challenge ahead would be to look at the wealth of the information collected from mechanical injuries in plants over the last few decades and probe the regeneration responses in natural growing conditions. Data collected from laboratory studies indicate that nature of wound alters the necessity of factors required for repair mechanisms [22,23].Thus whether the molecular players recruited in response to injury in laboratory and field conditions are similar or diverse await careful evaluation.

Conflict of interest statement

Nothing declared.

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