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REVIEW

Adventitious Root Regeneration: Molecular Basis and Influencing Factors

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ABSTRACT

Plant regeneration is a self-repair of the plant body in response to adverse conditions or damaged structures, and root regeneration allows the plant body to better adapt to its environment by supplementing the roots' structure. Previous research has shown that adventitious roots can be made to occur from scratch in two ways. Studies that simulate adventitious root regeneration through natural conditions allow the regeneration process to be broadly divided into three stages: the perception of early signals, the massive accumulation of auxin, and the transformation of cell fate. The strength of regeneration, in turn, is influenced by wounding, stress, hormones, etc. This study mainly reviews the molecular mechanisms of *de novo* adventitious roots and discusses how the environment, hormones, and its own development in *Arabidopsis thaliana*.

KEYWORDS

Arabidopsis thaliana; *de novo* adventitious root regeneration; phytohormone; regeneration molecular mechanism

1 Introduction

Plant regeneration is the process by which the plant body repairs or replaces damaged structures [1,2]. In contrast to animals, plants cannot move to withstand damage from adverse environments, so regeneration is an important ability for plants to resist and adapt to their environment [3,4]. The essence of plant regeneration is the process by which cells transform their cell fate after adverse, environmental stress [5], and totipotency or pluripotency is the basis of regeneration [3].

De novo adventitious roots is the root organogenesis after tissue injury and is also a form of plant regeneration. Plants have a strong ability to perceive external stimuli. In response to biological stress (such as invasion of pathogenic bacteria) or abiotic stress (such as high temperature, light or flood), plants can activate the expression regulation of ARFs (AUXIN RESPONSE FACTORs), ERFs (ETHYLENE RESPONSE FACTOR) and other related regulations so as to regenerate adventitious roots (ARs) to repair damaged organs [6–8]. Most plants can regenerate ARs indirectly from extraneous tissues and organs, or directly from various airborne organs [9]. For a long time, the common regeneration method in agriculture is to heal tissues and cuttings, such as detached leaves, hypocotyls, cuttings, etc., to produce ARs [10].

ARs are widely used in cutting and tissue culture of plant organs because they enlarge the root system of plants and enable plants and cells to regenerate [11]. In rice and other grains, ARs represents a major

component of the root system because the primary root RAM originating from the embryo is short-lived [12]. Some researchers prefer to refer to these types of ARs as dendritic crown roots because they are part of the normal developmental program of grains [12,13]. Compared with primary and lateral roots, ARs have no fixed location, are more influenced by environmental factors, and have a wider range of origins, such as isolated leaves, hypocotyls, and primary root wounds [14]. Although histological studies have shown that the formation of ARs begins in the primary cambium and its parenchyma cells, which are receptor cells for initiation of ARs [15], the lack of early molecular markers makes it much more difficult to identify protocells in ARs than in principal or lateral roots [16].

Phytohormone also play an important role in the regeneration of roots from nothing. The plant growth regulator auxin is a key regulator controlling rooting reaction, and the hormones cytokinin (CK), ethylene (ET), jasmonic acid (JA) and aurolactone generally reduce rooting ability [14,17–21]. As a result, a variety of hormones exist that tightly control and fine-tune the initiation and emergence of ARs. In tissue culture, the addition of growth hormone or CK can promote the regenerative ability of plant roots or shoots [22]. However, the key finding during tissue culture is that the ratio of added auxin and CK can determine cell fate, a high ratio of auxin promotes root regeneration, while a high ratio of CK promotes shoot regeneration [5]. Studies have confirmed that under natural conditions, plants can promote root regeneration from scratch by endogenous hormones on media without the addition of exogenous growth hormones [2,23].

According to the current research progress, the DNRR process can be roughly divided into three stages [2]: The first is the perception of early signals in isolated leaves, including damage signals and environmental signals such as light and temperature [24]. Accumulation of JA and ET caused by damage can stimulate the rooting of Arabidopsis explants, and dark treatment can promote the expression of *YUCCA 5/8/9 (YUC 5/8/9)* in leaf cells, resulting in the accumulation of auxin, thus promoting the formation of advents [2,25]. The second is the accumulation of auxin. Auxin, as a key plant hormone for *de novo* regeneration of ARs, will block rooting of leaf explants when its polar transport process is inhibited [26,27]. Finally, the fate of the cell changes. After auxin accumulation, ARF7/19 and other auxin response genes are activated to promote the transfer of auxin to regenerative cells and complete the transformation of cell fate [28,29]. With the participation of *WUSCHEL-RELATED HOMEOBOX5/7 (WOX5/7)* and *LATERAL ORGAN BOUNDARIES DOMAIN16/59 (LBD16/29)* genes, it promotes the regeneration of ARs [29,30].

Over the years, tissue culture techniques have been widely and well established in regeneration research, and some progress has been made in recent years in the cellular genealogy of the plant regeneration process and the molecular mechanisms controlling plant regeneration, Chen et al. studied the system of neoplastic root organogenesis and elucidated its regulatory mechanisms by culturing leaf explants from a plant, *Arabidopsis thaliana*, under model natural conditions [31]. Research on plant adventitious root regeneration has also significantly improved the efficiency of plant regeneration and transformation, and has been applied in the protection of endangered species and promotion of poplar clonal breeding program [32]. This paper reviews the molecular mechanisms of adventitious root formation, summarizing the process of cellular transition from response to a stimulus to fate. In addition, relevant factors that influence the presence or absence as well as the intensity of regeneration occurrences, such as wounding, phytohormones, and the developmental state of the plant itself, are summarized and discussed.

2 Adventitious Roots Regeneration

2.1 De novo Root Regeneration

De novo organ regeneration is the process by which plants regenerate ARs or adventitious shoots from isolated tissues and organs, with cellular totipotency being the basis of regeneration [33], such as *de novo* root regeneration (DNRR). To gain a more comprehensive understanding of DNRR, the process of regenerating roots from damaged leaves or hypocotyls has been explored in a medium that simulates natural conditions

[34]. The successful regeneration of ARs from isolated Arabidopsis leaves on a B5 medium without the addition of exogenous hormones confirmed the feasibility of this process occurring under natural conditions [15,27,31]. In addition, plant hormones play a key role in adventitious root regeneration, and growth hormone was found to accumulate rapidly near the wound after plant injury and is a key hormone affecting cell fate [35]. In addition, JA can also be activated rapidly after injury, mediating the activation of downstream signalling molecules, and is considered to be one of the response hormones [36].

2.2 De Novo Root Regeneration Pathway

There are two ways to regenerate adventitious rooting: one is the direct induction of adventitious root from isolated plant tissue or organs under suitable conditions, also known as the direct route. This process requires the involvement of endogenous plant Hormones, particularly the accumulation of growth hormones. Xu et al. demonstrated that the histological structure of Arabidopsis guaiac tissue is similar to that of the root primordium or root apical meristem in healing induction and that the intermediate cell layer is quiescent centre-like (QC-like), giving the plant organ the ability to regenerate ARs from scratch [37]. Root explants already have lateral root meristem tissue primordia along the somatic axis and can regenerate roots from the wound and near the primary root without callus inducing medium (CIM) pretreatment, whereas hypocotyl explants can only regenerate roots from the wound under these conditions [15,38]. *ERF115* can promote the accumulation of auxin near damaged cells and promote regeneration and tissue repair after tissue damage [35]. The second is the generation of ARs by a two-step method, in which isolated organs are generated on a healing CIM with the addition of exogenous hormones, which are then transferred to a root induction medium (RIM) to generate ARs [27,39,40], also known as the indirect route. Probably because of the pluripotent nature of the CIM, which produces root meristem-like pluripotent cell masses, followed immediately by the development of these cell masses on the RIM into specific root meristematic tissues [37,41]. In the intermediate cell layer, WOX5 directly interacts with PLETHORA1 and 2 (PLT1/2) [37], Promoting the expression OF TRYPTOPHAN AMINOTRANSFERASE OF Arabidopsis and generating endogenous auxin. WOX5 also interacts with type b Arabidopsis RESPONSE REGULATOR12 (ARR12) to inhibit type-a ARRs and break the negative feedback loop of CK signaling [27,37]. That is, by enhancing the production of auxin and the sensitivity of CK to promote the acquisition of pluripotency in the intermediate cell layer, and then differentiate into ARs.

3 Molecular Mechanism

Isolated damaged leaves of *Arabidopsis thaliana* can sense external signals and changes in their endogenous hormones, and regulate and express relevant signaling molecules. Although the application of healing tissues has been relatively common, the perception of external signals by plants, and the mechanisms of self-regulation caused by them, have been subject to in-depth study. In recent years, Xu et al. have systematically studied DNRR under simulated natural conditions and divided the process into three stages: early signal perception, massive accumulation of growth hormone, and cell fate transformation [2,39,42].

3.1 Phase I, Early Signaling

In the first stage, a variety of signals are sensed by isolated leaf explants, such as trauma, abiotic stresses and the plant's age. These stimuli influence the efficiency, speed and even the occurrence of regeneration of ARs in the plant. Early signals are sensed and can be transported to, for example, the chloroplast or some vascular cells [2]. After the leaf damage signal is sensed, it can cause rapid physicochemical changes and long-term hormonal signals to be activated [43]. First, Physical and chemical changes of intracellular Ca^{2+} concentration, membrane potential and reactive oxygen species [24,44]. After injury, the addition of a certain concentration of exogenous Ca^{2+} can promote the formation of adventitious roots, while the

addition of broad-spectrum Ca^{2+} channel inhibitors and Ca^{2+} chelating agents can inhibit the formation of AR induced by indoleacetic acid (IAA) or NO [45]. Hydrogen, a bioinert gas, plays an active role in adventitious root regeneration of cucumber explants [46]. Then hormones in the plant are activated, causing intercellular signalling molecules such as auxin, CK, JA, etc. [18,36,47–49]. As a plant hormone that responds quickly to injury, JASMONATE binds to the F-box protein receptor CORONATINE INSENSITIVE1 (COI1) within a few hours of damage to plant tissues or organs, promoting the binding of Jasmonate-Zim-Domain (JAZ) protein to COI1. It leads to JAZ ubiquitination and degradation, and activates ja signaling pathway [36,50]. After a few hours of damage, long-term signalling is activated accordingly, causing long-term signalling. The first is the activation of regeneration-related genes, such as the expression of the *YUC4* gene, which may be associated with the maintenance of plant growth hormone levels, in cells with a high regenerative capacity near plant injury [51,52]. After injury, (NAM/ATAF/CUC domain1) *NAC1* transcription factor gene can be specifically expressed in leaf explants, and play a role in the degradation of cell wall expansion protein by regulating the expression of *CEP* gene, and then participate in adventitious root regeneration through an auxin independent pathway [31]. In addition, the expression of some genes that affect the cellular environment can also positively regulate root regeneration, such as the Arabidopsis *ATAF1*, *ATAF2* and *CUC2* genes [31].

Different environmental signals can also be sensed by the plant and affect the efficiency of adventitious root regeneration to a greater or lesser extent. Light is one of the most important factors influencing adventitious root regeneration. Hypocotyl under alternating light and dark conditions. can be promoted by the interaction of HY5 (LONG HYPOCOTYL) and AGO1 with growth hormone-related response factors to regenerate its ARs [52]. Another gene related to growth hormone synthesis, YUC5/8/9, is expressed during dark treatment and enhances growth hormone synthesis and thus adventitious root regeneration. In addition, drought, nutrition and temperature can also be perceived by plants to affect regeneration efficiency [20,51,53].

The developmental state of the explant, such as age, is also an important factor in regeneration. The regenerative capacity of older leaves is significantly reduced compared to younger leaves, and the phenomenon of growth hormone accumulation during leaf regeneration was found in the study, interestingly, there was a significant increase in regeneration capacity when exogenous growth hormone was added to mature leaves, which also suggests that auxin is one of the factors affecting regeneration capacity [2]. The response caused by changes in the plant's state is complex, and the study of its regeneration mechanisms is important in breaking through the limits of regeneration. Early signals are sensed in time as the basis for regeneration and are coordinated by a variety of cellular tissues and organs, e.g., the cell wall can be sensed and transformed by molecules [54].

3.2 Phase II, Auxin Accumulation

Various plant hormones such as JA and abscisic acid (ABA) are involved in the regeneration of ARs from scratch, but the auxin is the key hormone in this process. Excessive amounts of growth hormone can lead to an increase in the number of ARs regenerated. In addition, the regeneration capacity of plants is significantly reduced when grown on media supplemented with the growth hormone biosynthesis inhibitors l-Kyn or yucasin or the growth hormone polar transport inhibitor naphthalic acid (NPA) [20,48]. Thus, growth hormone accumulation is a key step in the regulatory network of adventitious root regeneration signals, determining the presence or absence and intensity of regeneration.

Within hours of wound onset, plant hormones are activated for expression in response to stress signals. Growth hormone accumulates in transformed cells [55]. Tryptophan is a precursor substance for growth hormone biosynthesis, a process in which many genes are involved, such as those in the Arabidopsis tryptophan aminotransferase (TAA) family, the YUC family. YUC is highly sensitive to many early signals and the TAA gene can be continuously expressed before and after leaf isolation [26]. Thus, it has

been shown that environmental factors and short-term trauma signals can activate the expression of YUC1/4 and YUC5/8/9 [26]. Hydrolysis of IAA coupled with amino acids also contributes to the rapid production of auxin [26]. The response of ARF to auxin can promote the activation and response of downstream genes. Overexpressed LkARF7 and LkARF19 in *Larix kaempferi* can positively regulate LkBBM1 and promote the formation of adventitic roots [28]. Li et al. confirmed that PHYB can interact with INDOLE-ACETIC ACID14 (IAA14), ARF7, and ARF9. This interaction stabilizes IAA14 and inhibits transcriptional activity of ARF7 and ARF19, thereby inhibiting dark-induced hypocotyl adventitious root biogenesis. The hydrolysis of IAA coupled with amino acids also contributes to the rapid formation of auxin [44].

Auxin is transported by polar transport from the chloroplast to the vicinity of the wound. Studies have shown that auxin accumulates rapidly near the wound at around 12 h of wound onset, mainly in the procambium layer and nearby parenchymal cells, a region of cells also known as cells with regenerative potential [20,43,56]. The addition of NPA retains growth hormone in transformed cells and prevents accumulation into regeneration-receptive cells [31], confirming that auxin from regeneration-receptive cells is transported by transformed cells. In addition, the polar transport process of auxin is also influenced by other hormones and can be inhibited by gibberellin, which in turn inhibits adventitious root regeneration. In the DNRR process, auxin accumulation is a pivotal influence in undertaking signal perception and cell fate transformation.

3.3 Stage III: Cell Fate Transformation

The accumulated growth hormone is transported to cells with regenerative potential for the cell fate transition to occur. The protoplast and its surrounding thin-walled cells have been shown to have regenerative potential [2,4]. The cells with the regenerative potential of leaf explants can transform their cell fate and regenerate adventitious rooting under natural conditions or with the addition of specific hormones. Xu proposed four steps for cells to undergo a fundamental shift in fate: initiation, initiation, pattern, and emergence [2].

The first step is ‘initiation’, a process that occurs within about 1–2 days of cell injury when the transition from regenerative cells to root-establishing cells occurs and does not involve cell division. The expression of the stem cell regulators *WOX11* and *WOX12* was identified by CHIP-seq and others, which are subfamilies of the WOX intermediate branch and are functionally redundant [2]. Furthermore, deletion of *WOX11/12* leads to rooting defects, whereas overexpression of *WOX11/12* enhances the rooting capacity [15]. The occurrence of this process also marks the transition of regeneration potential cells to ARs initiation cells. *WOX11* is a direct target of the auxin signaling pathway [57]. During the regeneration of new roots, *WOX11* expresses and activates the expression of its target genes, links the upstream auxin signal with the downstream cell fate transition, initiates root and callus primordials, and regulates the establishment of original cells [57]. In addition, *WOX11/12* deficiency may lead to rooting defects, while overexpression of *WOX11/12* may enhance rooting ability [27]. This process also marks the transition from regenerative potential cells to adventitious root initiation cells.

Next is ‘initiation’, a process that takes place within 2–4 days of injury and, in addition to a cell fate transition, cell division occurs in response to growth hormone, resulting in the formation of a dome-shaped root primordium with multiple cell layers. During cell division, many cell cycle-related genes are activated [58–61] Studies have shown that *ERF115* acts as a CK signaling mechanism for AR initiation by activating CK, and *ERF115* can be activated by JA transcription, inhibiting AR initiation in a ninja dependent and independent manner [19,62]. At the same time, cell division may be inhibited by solanum lactone. During a fate transition in the apical meristem, the transcription factor *WOX11/12* activates the downstream *LBD16* and *WOX5/7* produces adventitious rooting primordia [15,27,63]. *LBD16* is essential in the lateral root, ARs and healing tissue formation. *lbd16* mutants showed significant defects in AR organogenesis in leaf explants, suggesting that *lbd16* is required for AR initiation [29,64]. This study was

proposed that *WOX11/12* directly activated the expression of *LBD16* in the process of adventitic root regeneration by explants of isolated leaves [15,64]. *WOX5/7* can also be considered a key gene in the stem cell niche [63,65,66]. As for the upstream regulatory mechanism controlling *WOX5* expression, Zhang et al. found that the suppressor of *WUSCHEL1* (*ROW1*), also known as *BARD1*, directly binds to the *WOX5* promoter region and restricts its transcription to QC [30]. It has been reported that WOX5/7 can interact with ARR12 and inhibit ARR5 to eliminate the negative feedback mechanism of CK signaling during callus induction, resulting in high CK sensitivity of explants. *WOX5* may also maintain the pluripotency of the intermediate cell layer of callus on CIM and promote the adventitia formation of callus on root-inducing medium (RIM) through high levels of growth hormone [37].

This is followed by 'moulding' and 'emergence', in which the root primordial cells undergo successive cell divisions to form the root apical meristem (RAM). The genes involved in this process are SHOOT ROOT (SHR) and SCARECROW ROOT (SCR) [67]. The root tip meristem gradually matures to form a root tip, which grows rapidly to break through the explant thus forming ARs. In conclusion, adventitious root regeneration from the tip is a cell fate transformation process that undergoes complex, sequential signalling regulation.

4 Influencing Factors

4.1 Developmental Constraints

The regeneration capacity of plants is closely related to their developmental state, with root regeneration capacity decreasing significantly as the plant ages. In general, the ability of mature plant explants to effectively regenerate branches is significantly lower than that of young branches [68]. The gradual maturation of plants reduces the regeneration capacity, which is a major obstacle to the asexual reproduction of good varieties, and part of this is related to phytohormone synthesis, with some studies showing that the conversion of peas from the nutritional mount state to the reproductive state is associated with reduced root regeneration, a corresponding reduction in auxin [69]. MiR156 is a factor associated with the regulation of plant development and can target the three SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors *SPL2*, *SPL10* and *SPL11* [62,68,70]. During the juvenile period, plants have a strong cell division and regeneration capacity. With age, miR156 synthesis decreases and inhibition of SPL targets is reduced [71,72]. SPL can directly inhibit Arabidopsis B-type response regulator activity and suppress shoot regeneration [68]. In addition, miR156 activity was reduced and the number of hypocotyls regenerated ARs was decreased, confirming that miR156 is also involved in adventitious rooting regeneration in Arabidopsis [73]. However, the mechanism of miR156/SPL in regulating adventitious rooting regeneration still needs to be studied in depth.

4.2 Environmental Constraints

4.2.1 Wound

Damage is the main trigger for adventitious root regeneration, by reactivating cell proliferation and reprogramming cells. Two molecular modes associated with injury: the cell wall derivative oligogalacturonide and extracellular ATP are sensed to elicit a cellular defence response [74–76]. Such as the translocation of calcium signals and the burst of reactive oxygen species [41]. The damage signal will be rapidly responded to by translocation of electrical and chemical signals to relevant signalling receptors [8], which can promote high expression of the key transcription factor APETALA2/ET response factor (AP2/ERF) to induce healing tissue formation [62,77]. In addition, WOUND-INDUCED DEDIFFERENTIATION1 (*WIND1*) directly upregulates the expression of the enhancer of SHOOT REGENERATION1 (*ESR1*), an Arabidopsis AP2/ERF transcription factor, to promote healing tissue formation and specific transformation [78].

Recent studies have shown that genes related to signalling such as JA, ET and ROS are rapidly mobilized after leaf isolation, while stress response, subcellular regulation and metabolism-related genes

are also mobilized to varying degrees, suggesting their involvement in cell fate transformation and root regeneration processes [39]. Analysis of trauma-induced transcriptome changes by RNA-seq data indicated that significant expression differences in 80% of gene expression profiles occur within 24 h after trauma [41]. Some genes encoding defence substance synthases, protein kinases that transduce calcium signals are more sensitive to trauma signals and activate expression rapidly within a short period of time [79]. After a period of time following the injury signal, genes associated with protein synthesis and cell proliferation are activated in response [39]. It is important to note that development, i.e., the environment, has a strong influence on the cellular protein synthesis capacity, and ribosomal transcript levels are significantly lower in mature organs than in areas of active cell proliferation [80]. These transcriptional dynamics may also affect endogenous hormone levels in traumatized organs. Hormone quantification and phenotypic analysis revealed that CK, a key hormone for healing tissue formation near the wound, is activated after injury and accumulates large amounts of tZ and cZ-type CKs [41]. Interestingly, the differences in the level of hormone activation response were highly significant under different physiological conditions. Wound-induced healing tissue formation strongly induced the CK synthesis-related genes *LOG1*, *LOG4*, *LOG7*, and *CYP735A2*, whereas there were no similar transcriptional changes in other wound inductions [81]. In addition, there is a significant accumulation of growth hormone during wound-induced ARs production, forming a growth hormone stream that promotes stem cell regeneration, whereas the role in healing tissue formation appears to be weak [35]. The coercive hormone ABA shows a significant inhibitory effect in both cell proliferation and healing tissue travel after injury [82].

AP2/ERFs are key transcriptional regulators of trauma induction, acting as receptors. Signals for trauma and activating the expression of downstream signals. Importantly, different groups of AP2/ERFs can trigger regenerative processes. Furthermore, in addition to trauma signalling plant hormones can also induce AP2/ERF promoters such as JA and salicylic acid (SA). GO analysis showed that *SPL10* can induce AP2/ERF to inhibit root regeneration by regulating the synthesis of growth hormone. In addition, SPL10 can also affect the hormone-stimulated rooting action of JA and SA [62]. Under different circumstances, regulatory factors can play different roles. Overall, it appears that trauma has two main effects: firstly, it forms a barrier to the flow of growth hormone, leading to the accumulation of growth hormone near the wound and to the concentration required for adventitious root regeneration [35,50]; secondly, it indirectly increases the efficiency of adventitious root regeneration by promoting the synthesis of auxin [50].

4.2.2 Light

Plant regeneration of ARs is influenced by various environmental conditions, such as nutrition, temperature and pH. Light is one of the most important factors affecting the regeneration of ARs from hypocotyls. In some cases, root or shoot regeneration can be inhibited by light [38]. In tissue culture experiments, light inhibited shoot regeneration in the first few hours after cotyledon tissue excision in *Arabidopsis thaliana*, while placing the explants in the dark promoted regeneration in only 2–6 h [38]. Light triggers the light signalling pathway and a key regulatory transcription factor downstream of light signalling, *ELONGATED HYPOCOTYL5* (*HY5*) and *AGO1*, can induce ARs production in the hypocotyl under alternating light and dark conditions, and the number of ARs in the hypocotyl of the mutant ago1 is reduced and the bound growth hormone content in the mutant ago1 is decreased as revealed by proteomic analysis [53], *ARF6* and *ARF8* have been reported to be downstream targets of miR160, and the number of ARs was reduced in the hypocotyls of mutants arf6 and arf8, so *ARF6* and *ARF8* may be positive regulators of adventitious root regeneration [49]. Recent studies have shown that photoreceptors can negatively regulate hypocotyl adventitious root production under dark conditions [4,53,55]. Under dark conditions, hypocotyl adventitious root regeneration is completely dependent on *ARF7*, *ARF19* and *LBD16*, and photoreceptor pigment B inhibits hypocotyl adventitious root formation by stabilizing *IAA14* and inhibiting *ARF7* and *ARF19* [44,53,55].

4.3 Hormone

4.3.1 JA

ARs in plants can come from non-root organs. In *Arabidopsis*, ARs can be formed from hypocotyls that complement their root structures, and in addition, isolated plant leaves can also produce ARs. Plant hormones play a key role in the development of new roots. JA, a trauma-inducing hormone, plays a different role in the regeneration of ARs from hypocotyls and isolated organs, respectively [83].

JA plays a positive regulatory role in the regeneration of ARs from isolated organs *in vitro* [50,80,83]. As a wound hormone, JA is rapidly induced and activated within a short time of leaf damage. It then directly activates the downstream target site ERF 109 and the activated *ERF109* can bind to the tryptophan biosynthesis precursor, *ANTHRANILATE SYNTHASE α1* (*ASA1*) gene, promoting its expression [6,84]. The binding of *ERF109* to the *ASA1* promoter has been demonstrated by chromatin immunoprecipitation (ChIP) [50], a process that also involves the tri-methylation on Lys27 of histone H3 (H3K36me3) enzyme [50,83]. In contrast, persistently high JA levels were detrimental to adventitious root regeneration, and after two hours, JA levels decreased and the JAZ protein interacted with *ERF109*, leading to inhibition of *ERF109* activity [50]. In addition, plant age also has an effect on adventitious root regeneration in isolated leaves. The miR156/SPL pathway in *Arabidopsis* plays a key role in senescence regulation [44,62,68,70,71]. SPL is a direct target site of miR156 and can be degraded upon binding by miR156. However, as leaves mature and senesce, miR156 content decreases and the ability to target SPL is diminished. The results of established studies confirm that *SPL2*, *SPL10* and *SPL11* are important regulatory genes in the regeneration of ARs from isolated leaves, negatively regulating *ERF109* and limiting the synthesis of growth hormone thereby inhibiting rooting [78,85,86]. Therefore, the onset of the regeneration process is also age-sensitive. Thus, trauma-induced JA is dynamically variable and regulates the regeneration of ARs in conjunction with growth hormone and epigenetics.

In addition to promoting the regeneration of ARs in isolated leaves, JA also inhibits the regeneration of ARs in the hypocotyl. However, JA does not regulate regeneration alone but interacts with growth hormone and CK to regulate the regeneration signalling pathway of ARs [33,87]. Auxin has a positive regulatory role in promoting the regeneration of ARs. Three transcription factors, ARF6, ARF8 and ARF17, mediate auxin signalling and control the expression of the enzymes GRETCHEN HAGEN3.3 (GH3.3), GH3.5 and GH3.6 [88]. The enzymes GH3.3, GH3.5 and GH3.6 catalyse the binding of free JA and indole-3-acetic acid (IAA) into amino acids and maintain homeostasis [88]. It was found that DIOXYGENASE FOR AUXIN OXIDATION 1 (DAO1) [86], an enzyme first identified in rice, controls a feedback pathway that stabilizes the crosstalk between growth hormone and JA during AR initiation [88]. CKs, on the other hand, inhibit ARs production and studies have found a decrease in CK content during the regeneration of ARs. CK oxidase/dehydrogenase 1 (CKX1) can degrade CK, and CKX1 activity is inhibited in the JA MYC2 signalling pathway. *ERF115* can act as one of the downstream target genes of JA and promotes CK expression. In addition, JA and CK may synergistically activate *APETALA2.6 LIKE* (*RAP2.6L*) (also known as *ERF 113*) expression. It has been shown that JA can inhibit root regeneration in different ways, Lakehal et al. proposes that JA signalling inhibits adventitious root regeneration in both a ninja-dependent and ninja-independent manner and that JA-induced ERF115 transcription factor inhibits regeneration in a CK-dependent manner [19]. Therefore, JA, auxin and CK synergistically negatively regulate adventitious root regeneration in the hypocotyl.

4.3.2 Auxin

As an important plant hormone, auxin plays a key role in the process of adventitious root regeneration. Feldmann et al. established a two-step method for indirect induction of *Arabidopsis* organ establishment by placing *Arabidopsis* explants in CIM and shoot-inducing medium (SIM) with different ratios of growth hormone and CKs [89], showing that organ regeneration from scratch requires the involvement of growth

hormone. In medium-simulating natural conditions, there was a significant accumulation of growth hormone in isolated leaves of *Arabidopsis* with elevated levels, indicating the possible involvement of growth hormone in the process of adventitious root regeneration [90,91]. In addition, when exogenous auxin was added to the medium, adventitious root regeneration was significantly increased, whereas the addition of growth hormone inhibitors reduced growth hormone accumulation and inhibited adventitious root regeneration [31,55,92].

As mentioned earlier in the mechanism of adventitious root regeneration, during the regeneration of ARs from isolated organs, when growth hormone accumulates, it reaches the vicinity of cells with regenerative potential through polar transport and reaches maximum growth hormone concentration, which in turn induces high expression of *WOX11* and *WOX12* in the forming layer and nearby thin-walled cells, marking the completion of the first fate transition from potential to root cells in head regeneration [15,37]. *WOX11* further activates the expression of *LBD16/29* [87], a downstream gene involved in lateral root formation. which is directly regulated by the growth hormone-responsive genes *AUXIN RESPONSE FACTOR 7* (*ARF7*) and *ARF19* and controls cell proliferation and loose cell wall arrangement during lateral root initiation [52,87]. LBD promotes cell division through cell division promotes the transformation of root mother cells into root primordial cells labelled by *WOX5/7*, marking the completion of the second cell fate transition, after which the primordial cells continue to grow and differentiate into a complete ARs [44,63]. Thus, the LBD family of genes plays an important role in the generation of ARs in response to auxin.

4.3.3 CK, ET

In addition to auxin and JA, plant hormones such as CK and ET are also involved in the process of adventitious root regeneration in plants. Exogenous application of CKs results in larger root tip meristems and CKs contribute to the specialization of the QC of the primary root tip. In the CK-deficient mutant background, *WOX5* is not specifically expressed in the resting centre of the primary root tip [93]. In *Arabidopsis* roots, there is an antagonistic effect of growth hormone and CK, whose minimal ratio is the boundary between cell division and differentiation and determines the fate of the cell [20,36]. The effect of CKs on regenerating roots is, on the one hand, through growth hormones, which affect their polar transport, and on the other hand, as downstream signalling molecules of the JA signalling pathway that inhibit root regeneration [20,36,93]. ET is also involved in the regeneration of ARs. When plant tissues are treated with ACC, a precursor substance of ET, the number of lateral roots is reduced and the transport of growth hormone is inhibited [94]. Moreover, the development of ARs is inhibited by the application of AVG or STS, inhibitors of ET synthesis [17].

The ability of plant regeneration reflects the high plasticity of plant cell fate. How to regenerate a single plant somatic cell into a complete plant has always been a very concerned problem in the field of plant research. It is the ability to regenerate ARs, adventitious buds, and even whole plants that enables tissue repair and survival in the face of harsh natural conditions or mechanical damage. Since the observation that plants have a strong regenerative ability, people began to develop and use them in agricultural production. Potatoes can be rapidly propagated by cutting, and fast-growing poplar trees commonly used in afforestation can be rapidly and economically planted by cuttings [32,95]. However, our understanding of the molecular mechanism is far from enough, and the technology and research method system are all obstacles for us to further explore the regeneration mechanism. For example, the mechanism of how plant cells respond to wounds and hormones to make the fate change, until the model plant *Arabidopsis thaliana* has the advantage of molecular genetic manipulation, we have entered a new era in the study of plant regeneration mechanism.

5 Discussion

During regeneration, external stimuli such as wounds and stresses ectopically activate the intrinsic developmental program and initiate the *ab initio* occurrence of adventitious root in the hypocotyl or isolated leaf of the plant. This is an orderly process from cells with regenerative potentials, such as formative layer cells, to the formation of root meristems, which is regulated by a highly complex network of signals through at least three successive stages, culminating in the transformation of cell fate to produce ARs (summarized in Fig. 1). Through clonal propagation by *in vitro* organs, a large number of offspring can be efficiently generated to save endangered plants, or high-quality clones that are genetically stable can be produced to produce disease-resistant and pest-resistant plants, which can also be used for genetically improved trees in planting and breeding programs [69], through comparing the genotypes of different rooting abilities of cutting, Quantitative trait loci (QTLS) related to the number of cuttings in Cottonwood (*Populus deltoides*), eucalyptus (*Eucalyptus* spp.) and poplar were identified [69]. However, how exactly does a cell perceive a stimulus and then break through epigenetic constraints to reprogram the cell? How are the factors involved at different stages of the transition coordinated and how are resources allocated? Are the regenerative mechanisms consistent across sites? Answers to these questions will provide a deeper understanding of adventitious root regeneration from scratch, which will have important practical applications in the regeneration of cuttings and healing tissues.

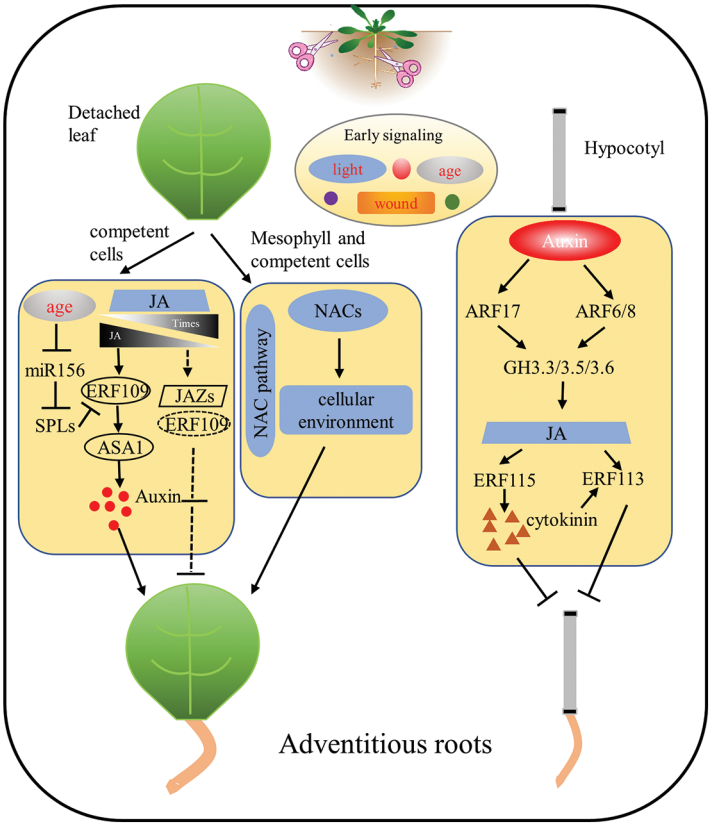

Figure 1: Molecular network of adventitious root regeneration in isolated organs of *Arabidopsis thaliana*. In the ARs process of isolated leaves, phytohormone (e.g., auxin, JA, CK) responses caused by wound signals and the age of the explant leaves simultaneously respond to regulate the ability of the explant to regenerate. In addition, NAC-related transcription factors are involved in adventitious root formation of detached leaves in a novel pathway. During hypocotyl adventitious root formation in Arabidopsis, auxin, JA and CK crosstalk with each other to form a molecular network for adventitious root formation in isolated organs

Much like animal regeneration, plant regeneration involves a variety of stem cells (or stem-like cells), Somatic embryogenesis is similar to dedifferentiation of animal somatic cells into induced pluripotent stem cells (iPS cells) or embryonic stem cells [13,96–99]. This dedifferentiation is easier in plant cells than in animal cells. The somatic cells of animals can achieve dedifferentiation by exogenous introduction of some specific genes or under certain conditions, but the frequency is very low. However, plant somatic cells can dedifferentiate efficiently under the induction of hormones or stress [13,96,97], so the research and development of plant regeneration will promote the progress in the field of regeneration. However, Among the factors affecting adventitious root development as well as efficiency, wounding, and state of its development, different phytohormones, and even different organs of the same hormone all have different effects. However, the regulation of adventitious root regeneration is more complex than we currently know, and there are many unanswered questions, such as the crosstalk between different signals, the critical points that trigger the response of different transcription factors, and how to balance the levels of different hormones to keep the plant in the most favourable state.

There are great differences in regeneration capacity among different species. The dicotyledonous model plant *Arabidopsis thaliana* is mostly used as the research object in the studies of adventitive root regeneration, and because the regenerative cells of *Arabidopsis thaliana* are all over the vascular system of leaves, it shows strong regenerative ability. However, for important food crops such as rice, wheat (*Triticum aestivum*) and corn (*Zea mays*), the model plants of monocotyledonous grasses, regenerative cells are limited to the base of immature leaves, so it is difficult to conduct tissue culture in mature tissues [12,45,100–102]. The explants usually used for monad cell regeneration are immature embryos, which are the main bottleneck for monad cell transformation, as these embryos need to be separated separately from the developing seed. As technology advances and develops, more research methods and tools are available for regeneration studies, such as single-cell RNA sequencing, which will give us more convenience and greater possibilities for future studies on adventitious root regeneration.

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