



Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.070>

PHYSIOLOGICAL ROLE OF ANTIOXIDANT ENZYMES IN ADVENTITIOUS ROOT DEVELOPMENT OF GRAPEVINE: A REVIEW

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(Date of Receiving-24-05-2026; Date of Revision-30-07-2026; Date of Acceptance-20-08-2026)

ABSTRACT

Adventitious root (AR) formation is a fundamental biological process that underpins vegetative propagation of grapevine (*Vitis vinifera* L.), directly influencing nursery production, vineyard establishment, and multiplication of elite cultivars and rootstocks. Although exogenous auxins are routinely employed to enhance rooting, successful AR development depends on a complex regulatory network involving hormonal signalling, reactive oxygen species (ROS), antioxidant defence mechanisms, and phenolic metabolism. Recent studies have demonstrated that ROS function not only as by-products of oxidative metabolism but also as essential signalling molecules that regulate cell dedifferentiation, root primordium initiation, and subsequent root emergence. However, excessive ROS accumulation disrupts cellular homeostasis and impairs rooting, necessitating a tightly regulated antioxidant system. Enzymes such as superoxide dismutase, catalase, peroxidases, ascorbate peroxidase, and glutathione reductase maintain redox balance by modulating ROS concentrations, thereby ensuring an optimal oxidative environment for root development. Simultaneously, phenolic enzymes, particularly phenylalanine ammonia-lyase and polyphenol oxidase, regulate phenolic metabolism, influence auxin stability, participate in lignification, and contribute to wound healing and root induction. Despite substantial advances in understanding these individual pathways, their coordinated interactions during grapevine adventitious rooting remain incompletely understood. This review critically synthesizes current knowledge on physiological, biochemical, and molecular roles of antioxidant and phenolic enzymes in AR formation, integrating evidence from grapevine and other woody perennial fruit crops. Particular emphasis is placed on crosstalk among auxin signalling, ROS homeostasis, antioxidant defense, and phenolic metabolism that collectively governs rooting competence. Finally, current knowledge gaps and emerging research opportunities, including omics-based approaches and molecular breeding strategies, are discussed to provide future directions for improving propagation efficiency and nursery performance in grapevine.

Key words : *Vitis vinifera*, Adventitious rooting, Reactive oxygen species, Antioxidant enzymes, Redox signalling.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the world's most economically important fruit crops, propagated almost exclusively through vegetative methods to preserve cultivar characteristics and exploit stress-tolerant rootstocks. Efficient adventitious root (AR) formation in stem cuttings is therefore essential for nursery production and sustainable vineyard establishment (Hartmann *et al.*, 2018; Druege *et al.*, 2019).

Unlike embryonic roots, adventitious roots arise from nonroot tissues following stem cutting excision through wound perception, cellular dedifferentiation, founder cell specification, root primordium formation, and vascular reconnection. Rooting efficiency is influenced by genotype, hormone balance, carbohydrate availability, environmental conditions, and cellular redox homeostasis. Although indole-3-butyric acid (IBA) is widely used to enhance rooting, considerable variation among grapevine

cultivars and rootstocks indicates that additional physiological and biochemical mechanisms regulate rooting competence (Bellini *et al.*, 2014; Steffens and Rasmussen, 2016; Roussos, 2018; Köse *et al.*, 2011).

Reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), are now recognized as important signalling molecules regulating cell division, differentiation, vascular development, and root organogenesis. Following cutting excision, transient ROS accumulation interacts with auxin to initiate developmental reprogramming, whereas excessive ROS causes oxidative damage, highlighting the importance of maintaining redox homeostasis (Mittler, 2017; Baxter *et al.*, 2014; Noctor *et al.*, 2018). This balance is achieved through antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and class III peroxidases, whose activities closely correspond with successive stages of adventitious rooting (Köse *et al.*, 2011; Porfírio *et al.*, 2016; Zhang *et al.*, 2019).

Phenolic metabolism provides an additional layer of regulation by protecting endogenous indole-3-acetic acid (IAA), regulating peroxidase activity, and contributing to cell wall remodelling and lignification during root development. Together with recent molecular studies identifying AUXIN RESPONSE FACTORS (ARFs), LATERAL ORGAN BOUNDARIES DOMAIN (LBD) proteins, WUSCHEL RELATED HOMEODOMAIN (WOX) genes, PIN FORMED (PIN) transporters, microRNAs, and NADPH oxidases, these findings demonstrate that auxin signalling, ROS, antioxidant enzymes, and phenolic metabolism function as an integrated regulatory network controlling adventitious rooting (Goufo *et al.*, 2020; Dixon and Paiva, 1995; Guan *et al.*, 2015; Li, 2021; Liu *et al.*, 2014).

Despite these advances, coordinated roles of antioxidant enzymes and phenolic metabolism during grapevine adventitious rooting remain poorly understood, with most mechanistic knowledge derived from model plants and other woody species. This review synthesizes current physiological, biochemical, and molecular evidence on the roles of ROS, antioxidant enzymes, and phenolic metabolism in grapevine adventitious rooting, identifies key knowledge gaps, and highlights future research directions for improving vegetative propagation and nursery production.

Physiological and developmental regulation of Adventitious Root formation

Adventitious root (AR) formation is a post-embryonic developmental process that enables plants to

regenerate roots from non-root tissues in response to developmental or environmental cues. In grapevine, AR formation underpins vegetative propagation through stem cuttings and is essential for nursery success and vineyard establishment. Unlike lateral roots, adventitious roots arise from differentiated stem tissues that acquire rooting competence following wounding and hormonal stimulation, reflecting remarkable developmental plasticity of plants (Bellini *et al.*, 2014; Steffens and Rasmussen, 2016).

Stem cutting excision triggers rapid physiological and metabolic reprogramming, including disruption of vascular continuity, changes in carbohydrate transport, hormone distribution, and cellular redox status. These responses activate signaling pathways that promote tissue repair and root induction. Among them, auxin redistribution to the basal region is one of the earliest and most critical events, explaining the widespread use of indole-3-butyric acid (IBA) to enhance rooting in grapevine propagation (Hartmann *et al.*, 2018 and Druege *et al.*, 2019).

Adventitious rooting proceeds through four overlapping stages: induction, initiation, root primordium formation, and root emergence. During induction, cells perceive wound and hormonal signals and undergo metabolic reprogramming. This is followed by renewed cell division in cambial or parenchymatous tissues, formation of root initials, development of root primordia, vascular reconnection, and finally emergence of functional roots (Bellini *et al.*, 2014 and Guan *et al.*, 2015).

Auxin is principal regulator of these stages through AUXIN RESPONSE FACTOR (ARF) mediated gene expression controlling founder cell specification and meristem development. However, rooting depends on hormonal crosstalk in which cytokinins generally inhibit rooting, ethylene and jasmonic acid enhance auxin mediated responses and gibberellins and abscisic acid exert context dependent effects (da Costa *et al.*, 2013; Guan *et al.*, 2015; Li, 2021). Adequate carbohydrate reserves are equally important because they provide both energy and signaling molecules required for cell division and root development, with cuttings containing higher carbohydrate levels generally exhibiting superior rooting capacity (Druege *et al.*, 2019 and Ahkami *et al.*, 2013).

Rooting competence varies considerably among grapevine cultivars and rootstocks owing to differences in hormone balance, carbohydrate metabolism, phenolic composition, and oxidative metabolism. Genotypes with superior rooting ability display characteristic changes in antioxidant enzyme activities during root induction, indicating that rooting depends on coordinated metabolic regulation rather than auxin alone (Köse *et al.*, 2011).

Environmental factors, including temperature, humidity, oxygen availability, light and substrate conditions, further influence rooting by affecting hormone balance, respiration, and carbohydrate utilization. Controlled propagation environments therefore improve rooting efficiency by supporting active metabolism while minimizing stress (Hartmann *et al.*, 2018 and Roussos, 2018).

Increasing evidence indicates that successful adventitious rooting depends on the integration of hormonal signaling with cellular redox regulation. Wound induced reactive oxygen species interact with auxin signaling to regulate cell fate determination, root meristem establishment, and primordium development, while antioxidant enzymes and phenolic metabolism maintain redox homeostasis and prevent oxidative damage. These interconnected mechanisms provide physiological basis for adventitious root formation and form the foundation for subsequent discussion on ROS mediated regulation.

Reactive Oxygen Species as Developmental signals during Adventitious Root formation

Reactive oxygen species (ROS) are central regulators of adventitious root (AR) formation, functioning as signaling molecules rather than merely harmful byproducts of aerobic metabolism. Following cutting excision, controlled production of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) initiates signaling pathways that regulate gene expression, hormone homeostasis, cell cycle reentry, and tissue differentiation (Table 1). Successful rooting therefore depends on maintaining ROS within a physiological range that supports signaling without causing oxidative damage (Mittler, 2017 and Noctor *et al.*, 2018).

Stem cutting excision induces a rapid oxidative burst mediated by respiratory burst oxidase homologs (RBOHs), cell wall peroxidases, and organelles including chloroplasts, mitochondria, and peroxisomes. Among ROS, H_2O_2 plays a key signaling role because of its stability, membrane permeability, and ability to coordinate local wound responses with developmental processes (Baxter *et al.*, 2014; Waszczak *et al.*, 2018). ROS closely interact with auxin signaling, as auxin accumulation at cutting base stimulates ROS production, while H_2O_2 enhances auxin-responsive gene expression, forming a feedback loop that promotes root induction. Inhibition of either auxin transport or ROS production markedly reduces adventitious rooting, demonstrating their interdependence (Li *et al.*, 2009 and Guan *et al.*, 2015).

The concept of the oxidative window explains the dual role of ROS during rooting. Insufficient ROS fail to

activate developmental signaling, whereas excessive accumulation causes lipid peroxidation, protein oxidation, and programmed cell death. Antioxidant enzymes therefore regulate ROS within an optimal range required for founder cell specification and root meristem initiation, making them active participants in developmental regulation rather than simple detoxification systems (Mittler, 2017 and Roussos, 2023).

Reactive oxygen species also contribute to cell wall remodeling required for root primordium development and emergence. Hydroxyl radicals increase cell wall extensibility, while class III peroxidases regulate balance between cell wall loosening and lignification. In addition, ROS interact with nitric oxide (NO), calcium ions (Ca^{2+}) and mitogen activated protein kinase (MAPK) cascades to coordinate auxin signaling, antioxidant metabolism, and transcriptional regulation during root development (Cosio and Dunand, 2009; Tsukagoshi, 2016; Roussos, 2023; Li, 2021).

Evidence from woody species supports developmental role of ROS. In poplar, transient H_2O_2 accumulation coincided with activation of genes involved in auxin transport, cell cycle regulation, and root meristem development, whereas inhibition of ROS reduced rooting efficiency (Zhang *et al.*, 2019). Similar temporal changes in oxidative metabolism have been reported in olive (Porfirio *et al.*, 2016). Although direct studies in grapevine remain limited, comparative analyses of rootstocks indicate that superior rooting ability is associated with balanced oxidative metabolism and coordinated antioxidant responses (Köse *et al.*, 2011). Collectively, these findings establish ROS as central developmental regulators integrating wound signaling, hormonal regulation, and cellular differentiation during adventitious rooting, thereby highlighting the essential role of antioxidant enzymes in maintaining redox homeostasis during root formation.

Antioxidant enzymes in Adventitious Root formation

Reactive oxygen species (ROS) generated during adventitious root (AR) formation require precise regulation to maintain cellular redox homeostasis. Although ROS function as essential developmental signals, excessive accumulation damages membranes, proteins, and nucleic acids. Therefore, successful rooting depends on maintaining ROS within an optimal physiological range through coordinated antioxidant activity rather than complete ROS elimination (Mittler, 2017; Noctor *et al.*, 2018).

The enzymatic antioxidant system includes superoxide dismutase (SOD), catalase (CAT), class III peroxidases

(POX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR). These enzymes function cooperatively, particularly through ascorbate glutathione cycle, to regulate ROS production and scavenging while preserving their signaling functions (Gill and Tuteja, 2010; Foyer and Noctor, 2011).

Superoxide Dismutase

Superoxide dismutase is the first enzymatic defense against oxidative stress, catalyzing the conversion of superoxide radicals into hydrogen peroxide and oxygen. This reaction limits oxidative damage while generating H_2O_2 , an important signaling molecule during root development. Increased SOD activity is commonly observed during the induction phase, reflecting enhanced wound responsive metabolism and founder cell specification. As rooting progresses, SOD activity generally declines, indicating stage specific regulation (Gill and Tuteja, 2010; Porfírio *et al.*, 2016; Li *et al.*, 2009). In grapevine, rootstocks with superior rooting ability exhibit more efficient oxidative metabolism during induction, suggesting that controlled superoxide metabolism contributes to rooting competence (Köse *et al.*, 2011).

Catalase

Catalase rapidly converts hydrogen peroxide into water and oxygen, preventing excessive H_2O_2 accumulation while maintaining concentrations required for signaling. Catalase activity is generally low during root induction to permit transient H_2O_2 accumulation and increases during primordium differentiation to restore redox balance and protect actively dividing tissues (Mittler, 2017; Porfírio *et al.*, 2016; Gill and Tuteja, 2010).

Class III Peroxidases

Class III peroxidases regulate ROS homeostasis while participating in phenolic oxidation, auxin catabolism, lignification and cell wall remodeling. Their activity is developmentally regulated, remaining relatively low during induction to preserve endogenous auxin and increasing during root primordium formation to promote vascular differentiation and structural strengthening (Cosio and Dunand, 2009; da Costa *et al.*, 2013). In grapevine, contrasting rootstocks display distinct temporal patterns of peroxidase activity associated with rooting performance, while similar observations in olive support conserved role of peroxidases during adventitious rooting in woody species (Köse *et al.*, 2011; Macedo *et al.*, 2013; Porfírio *et al.*, 2016).

Ascorbate Glutathione Cycle

The ascorbate glutathione cycle regulates intracellular

hydrogen peroxide through the coordinated action of APX, MDHAR, DHAR and GR. APX reduces H_2O_2 using ascorbate, whereas MDHAR, DHAR and GR regenerate reduced ascorbate and glutathione, sustaining antioxidant capacity. Compared with catalase, APX regulates lower H_2O_2 concentrations associated with developmental signaling (Foyer and Noctor, 2011). Although information in grapevine is limited, studies in poplar, mung bean, and other woody species consistently report increased APX and GR activities during root induction, emphasizing importance of this pathway in maintaining favorable redox conditions during early root development (Li *et al.*, 2009 and Zhang *et al.*, 2019).

Overall, antioxidant enzymes function as active regulators of developmental signaling rather than simple detoxification systems. Their coordinated temporal regulation controls intensity and duration of ROS signals required for successful adventitious rooting, while their interaction with phenolic metabolism provides an additional layer of regulation discussed in the following section.

Phenolic Metabolism and Phenolic enzymes in Adventitious Root formation

Phenolic compounds are integral components of the biochemical network regulating adventitious root (AR) formation. Besides their antioxidant functions, phenolics influence root induction by interacting with auxin metabolism, reactive oxygen species (ROS), cell wall modification, and lignification. Thus, balance between phenolic biosynthesis, oxidation, and polymerization is a key determinant of rooting competence in woody species, including grapevine (Dixon and Paiva, 1995; Goufo *et al.*, 2020).

The phenylpropanoid pathway, initiated by phenylalanine ammonia lyase (PAL), produces diverse phenolic compounds such as phenolic acids, flavonoids, tannins, lignin precursors, and stilbenes. These metabolites scavenge ROS generated after cutting excision, protect endogenous indole-3-acetic acid (IAA) from oxidative degradation, and modulate oxidative enzymes involved in auxin catabolism, thereby creating favorable conditions for root induction (Dixon and Paiva, 1995; Cosio and Dunand, 2009). Following wounding, soluble phenolics accumulate at cutting base, reducing oxidative damage, stabilizing cellular membranes and enhancing auxin availability. As rooting progresses, phenolic metabolism shifts toward lignification and vascular differentiation required for root emergence (Porfírio *et al.*, 2016).

Among phenolic enzymes, PAL catalyzes first committed step of the phenylpropanoid pathway and is consistently associated with wound healing, phenolic

accumulation, and improved rooting. Although studies in grapevine are limited, research in olive, walnut, and other woody species demonstrates a positive relationship between PAL activity and root initiation (Porfírio *et al.*, 2016; Cheniany *et al.*, 2010). Polyphenol oxidase (PPO) regulates phenolic oxidation, contributing to wound healing and cell wall strengthening while exhibiting stage specific activity that preserves phenolics during induction and increases during root emergence (Macedo *et al.*, 2013; Köse *et al.*, 2011). Similarly, class III peroxidases link antioxidant and phenolic metabolism by regulating phenolic oxidation, lignification, vascular differentiation, and auxin metabolism, with their effects depending on the developmental stage (Cosio and Dunand, 2009; Porfírio *et al.*, 2016).

Grapevine accumulates a wide range of phenolic metabolites, including hydroxycinnamic acids, flavonoids, anthocyanins, and stilbenes such as resveratrol. Although extensively studied in fruit quality and defense, their role in vegetative propagation remains poorly understood. Genotype dependent differences in phenolic composition may partly explain variation in rooting ability, with superior rootstocks maintaining more balanced oxidative metabolism and phenolic profiles during root induction (Goufo *et al.*, 2020 and Köse *et al.*, 2011).

Phenolic compounds also cooperate with antioxidant enzymes to maintain oxidative window required for developmental signaling. While SOD, CAT and APX enzymatically regulate ROS, low molecular weight phenolics directly scavenge free radicals and stabilize cellular membranes, together forming an integrated antioxidant network (Gill and Tuteja, 2010; Noctor *et al.*, 2018). Despite growing evidence, regulatory role of individual phenolic metabolites during grapevine adventitious rooting remains poorly understood, highlighting the need for integrated metabolomic, transcriptomic, and biochemical studies to identify key phenolic regulators of rooting competence (Table 2).

Crosstalk among Auxin, Reactive Oxygen species, Antioxidant Enzymes and Phenolic Metabolism during Adventitious Root formation (Fig. 1)

Adventitious root (AR) formation is regulated by an integrated network involving auxin, reactive oxygen species (ROS), antioxidant enzymes, and phenolic metabolism rather than by individual pathways. Auxin initiates root induction after cutting excision through basipetal transport and local accumulation of indole-3-acetic acid (IAA) or application of indole-3-butyric acid (IBA). However, its activity is closely coordinated with ROS, antioxidant metabolism, and phenolic compounds,

which collectively regulate cell fate, meristem establishment, tissue differentiation, and root emergence (Druege *et al.*, 2019 and Li, 2021).

Auxin stimulates localized ROS production through respiratory burst oxidase homologs (RBOHs), while ROS enhance auxin transport, signaling, and expression of auxin responsive genes, creating a positive feedback loop that drives developmental reprogramming (Guan *et al.*, 2015; Li, 2021). At molecular level, this interaction involves AUXIN RESPONSE FACTORS (ARFs), LATERAL ORGAN BOUNDARIES DOMAIN (LBD) proteins, WUSCHEL RELATED HOMEBOX (WOX) transcription factors and PIN FORMED (PIN) auxin transporters, which together regulate founder cell specification, root primordium initiation, and meristem development (Liu *et al.*, 2014 and Li, 2021).

Antioxidant enzymes provide a critical regulatory interface by maintaining ROS within oxidative window required for developmental signaling. Superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and class III peroxidases regulate intensity and duration of ROS accumulation, thereby influencing auxin signaling, cell division, and tissue differentiation (Mittler, 2017; Noctor *et al.*, 2018). Phenolic compounds further strengthen this network by scavenging excess ROS, protecting endogenous IAA from oxidative degradation, and regulating peroxidase mediated cell wall remodeling, lignification and vascular differentiation (Dixon and Paiva, 1995; Porfírio *et al.*, 2016).

Evidence from woody species supports this integrated model. In olive, coordinated changes in phenolic accumulation, peroxidase activity, and auxin levels accompany root primordium development, while transcriptomic studies in poplar demonstrate coordinated expression of ROS responsive genes, auxin transporters, and antioxidant enzymes during root induction (Porfírio *et al.*, 2016; Zhang *et al.*, 2019). Although, comparable molecular studies in grapevine remain limited, rootstocks with superior rooting ability exhibit coordinated regulation of oxidative enzymes and phenolic metabolism, suggesting similar mechanisms operate in grapevine (Köse *et al.*, 2011; Goufo *et al.*, 2020).

Overall, current evidence supports a model in which auxin acts as primary developmental trigger, ROS function as secondary messengers, antioxidant enzymes regulate redox homeostasis and phenolic compounds stabilize both ROS and auxin metabolism. Integrating physiological studies with transcriptomics, metabolomics, and functional genomics will further clarify these interactions and facilitate the development of improved propagation

Table 1 : Major antioxidant enzymes involved in adventitious root formation and their physiological functions.

Enzyme	Primary reaction	Physiological role during adventitious rooting	Predominant stage of activity	Representative studies
Superoxide dismutase (SOD)	Converts superoxide radicals ($O_2^{\bullet-}$) into H_2O_2 and O_2	Initiates antioxidant defense, regulates ROS signaling, prevents oxidative damage, generates H_2O_2 for developmental signaling	Root induction	Li <i>et al.</i> (2009), Köse <i>et al.</i> (2011), Porfírio <i>et al.</i> (2016)
Catalase (CAT)	Converts H_2O_2 into H_2O and O_2	Controls hydrogen peroxide accumulation, prevents oxidative stress, restores redox balance	Root initiation and primordium development	Gill and Tuteja (2010), Porfírio <i>et al.</i> (2016)
Ascorbate peroxidase (APX)	Reduces H_2O_2 using ascorbate	Fine regulation of intracellular H_2O_2 , protection of actively dividing cells	Induction to primordium formation	Foyer and Noctor (2011), Zhang <i>et al.</i> (2019)
Glutathione reductase (GR)	Regenerates reduced glutathione (GSH)	Maintains glutathione pool, supports redox homeostasis through ascorbate–glutathione cycle	Throughout rooting process	Foyer and Noctor (2011), Zhang <i>et al.</i> (2019)
Monodehydroascorbate reductase (MDHAR)	Regenerates ascorbate from monodehydroascorbate	Sustains antioxidant capacity and ROS detoxification	Active cell division	Foyer and Noctor (2011)
Dehydroascorbate reductase (DHAR)	Regenerates reduced ascorbate using glutathione	Maintains cellular antioxidant pool and oxidative balance	Active rooting stages	Foyer and Noctor (2011)
Class III peroxidases (POX)	Oxidizes phenolic substrates using H_2O_2	Regulates ROS homeostasis, auxin catabolism, lignification, vascular differentiation and cell wall remodeling	Root initiation, primordium formation and emergence	Cosio and Dunand (2009), Köse <i>et al.</i> (2011), Macedo <i>et al.</i> (2013), Porfírio <i>et al.</i> (2016)

strategies for grapevine.

Molecular Regulation of Antioxidant and Phenolic pathways during Adventitious Root formation

Advances in molecular biology have revealed that adventitious root (AR) formation is controlled by integrated gene regulatory networks coordinating auxin signaling, reactive oxygen species (ROS), antioxidant enzymes, phenolic metabolism, and developmental gene expression. Transcriptomic and functional genomic studies demonstrate that these pathways function cooperatively to determine rooting competence, providing new opportunities to improve grapevine propagation through molecular and biotechnological approaches (Bellini *et al.*, 2014 and Li, 2021).

Auxin responsive transcription factors are central regulators of root initiation. Following auxin accumulation, AUXIN RESPONSE FACTORS (ARFs), particularly ARF6 and ARF8, promote founder cell specification and root meristem establishment, whereas ARF17 acts as a negative regulator. Their activity is further controlled by microRNAs, especially miR160 and miR167 (Bellini *et al.*, 2014; Li, 2021). Downstream, LATERAL ORGAN BOUNDARIES DOMAIN (LBD) transcription factors, including LBD16, LBD18, and LBD29, regulate cellular dedifferentiation, cell cycle reentry, and root primordium formation, while WUSCHEL RELATED HOMEBOX (WOX) genes, particularly WOX11, WOX12, and WOX5, control founder cell specification and root meristem maintenance (Guan *et al.*, 2015; Li, 2021; Liu *et al.*, 2014).

Reactive oxygen species regulate gene expression through redox sensitive signaling involving mitogen activated protein kinases (MAPKs), calcium dependent protein kinases (CDPKs), and transcription factors that coordinate antioxidant defense, auxin transport, cell wall remodeling, and phenylpropanoid metabolism (Mittler, 2017 and Waszczak *et al.*, 2018). Transcriptomic studies also show stage specific expression of antioxidant enzyme genes encoding

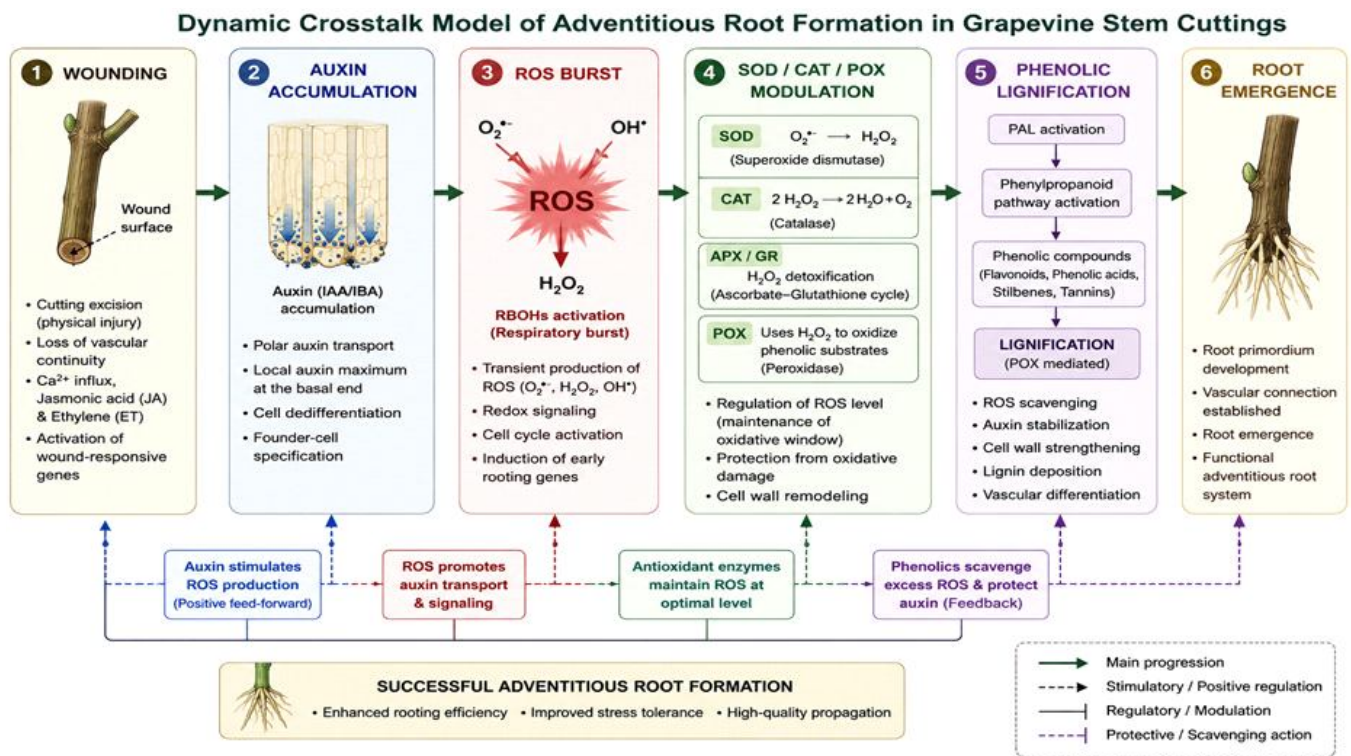


Fig. 1 : Dynamic crosstalk model illustrating the sequential and interactive regulation of adventitious root formation in grapevine stem cuttings.

superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione S transferases (GSTs), and class III peroxidases, enabling precise regulation of ROS during root induction. In poplar, coordinated upregulation of ROS responsive genes and antioxidant enzymes confirms that redox regulation occurs at both transcriptional and biochemical levels (Zhang *et al.*, 2019).

Genes involved in phenylpropanoid pathway, including phenylalanine ammonia lyase (PAL), cinnamate 4 hydroxylase (C4H), and 4 coumarate CoA ligase (4CL), are similarly induced during rooting, promoting phenolic biosynthesis for antioxidant defense, auxin stabilization, lignification, and vascular differentiation (Dixon and Paiva, 1995; Porfírio *et al.*, 2016). Although molecular studies in grapevine remain limited, available evidence indicates that superior rootstocks differentially express genes associated with auxin signaling, oxidative metabolism, and secondary metabolism. Future integration of transcriptomics, proteomics, metabolomics and genome editing technologies such as CRISPR/Cas will facilitate identification of key regulatory genes and molecular markers for improving rooting efficiency in grapevine (Zhang *et al.*, 2019).

Future Perspectives and Research priorities

Despite significant advances in understanding physiological, biochemical and molecular regulation of

adventitious root (AR) formation, important knowledge gaps remain, particularly in grapevine. Most current understanding of reactive oxygen species (ROS), antioxidant enzymes and phenolic metabolism is based on model plants or a few woody species such as poplar, olive, apple, and tea. Because rooting responses are highly genotype dependent, future research should emphasize grapevine-specific studies integrating physiological, biochemical, molecular, and omics approaches to clarify mechanisms regulating rooting competence.

Emerging technologies such as precision agriculture and artificial intelligence (AI) provide new opportunities for propagation research. Automated phenotyping using hyperspectral imaging, chlorophyll fluorescence, thermal imaging, and machine learning can monitor rooting non destructively, predict rooting success, and enable high throughput screening of genotypes and propagation treatments, improving nursery efficiency and reducing production costs.

Future studies should also adopt integrated, multidisciplinary approaches that simultaneously examine hormonal regulation, oxidative metabolism, phenolic biosynthesis, carbohydrate allocation and gene expression. Combining plant physiology, biochemistry, molecular biology, metabolomics, computational biology and horticultural science will advance understanding of root developmental biology and support development of more

Table 2 : Phenolic enzymes and phenolic metabolites associated with adventitious root formation.

Component	Major function	Role in adventitious rooting	Representative studies
Phenylalanine ammonia-lyase (PAL)	First enzyme of phenylpropanoid pathway	Initiates phenolic biosynthesis, enhances antioxidant capacity, protects endogenous auxin, promotes wound healing	Dixon and Paiva (1995), Cheniany <i>et al.</i> (2010), Porfírio <i>et al.</i> (2016)
Polyphenol oxidase (PPO)	Oxidation of phenolic compounds to quinones	Wound sealing, defense response, regulation of phenolic availability, cell wall strengthening	Macedo <i>et al.</i> (2013), Köse <i>et al.</i> (2011)
Class III peroxidase (POX)	Oxidative polymerization of phenolics	Lignification, vascular differentiation, phenolic oxidation, ROS regulation	Cosio and Dunand (2009), Porfírio <i>et al.</i> (2016)
Phenolic acids (caffeic, ferulic, p-coumaric acids)	Antioxidant metabolites	Scavenge ROS, stabilize auxin, reduce oxidative injury	Dixon and Paiva (1995), Goufo <i>et al.</i> (2020)
Flavonoids	Secondary metabolites with antioxidant activity	Modulate auxin transport, regulate ROS homeostasis, protect developing tissues	Goufo <i>et al.</i> (2020), Li (2021)
Stilbenes (e.g., resveratrol)	Grapevine-specific defense compounds	Antioxidant activity, stress adaptation, possible regulation of rooting competence	Goufo <i>et al.</i> (2020)
Lignin precursors (monolignols)	Structural polymers	Promote vascular differentiation and strengthen emerging root tissues	Dixon and Paiva (1995), Cosio and Dunand (2009)
Condensed tannins	Polymeric phenolics	Antioxidant protection and cellular defense during wound healing	Goufo <i>et al.</i> (2020)

efficient and sustainable propagation technologies for grapevine and other woody fruit crops.

Conclusion

Adventitious root formation is a complex developmental process regulated by coordinated interaction of hormonal, biochemical, and molecular pathways rather than by auxin alone. Wound induced auxin accumulation triggers a transient reactive oxygen species (ROS) burst that functions as a developmental signal, while antioxidant enzymes including superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and class III peroxidases maintain ROS within an optimal oxidative window required for root induction and development. Simultaneously, phenolic metabolism contributes to antioxidant defense, auxin stabilization, wound healing, lignification, and vascular differentiation, thereby linking oxidative metabolism with structural development. Together, auxin, ROS, antioxidant enzymes, and phenolic compounds form an integrated regulatory network that governs founder cell specification, root primordium formation and adventitious root emergence. Despite significant advances, the molecular regulation of antioxidant and phenolic pathways during grapevine rooting remains insufficiently understood, highlighting the need for grapevine specific studies integrating physiology

with transcriptomics, metabolomics, proteomics, genome editing and systems biology. Such multidisciplinary approaches will facilitate identification of key regulatory genes and biochemical markers associated with rooting competence, ultimately improving propagation efficiency, nursery production and supporting the sustainable expansion of viticulture under changing environmental conditions.

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