Molecular mechanisms regulating storage root formation in plants

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Abstract*— *Storage roots are important for the growth and development in plants because they provide nutrients, water, and energy storage. Storage roots are also modulating growth direction, disease resistance, and root formation at the cellular and molecular level through interactions of genes and gene networks. However, molecular mechanisms regulating storage root formation in plants are not fully understood. In this review, we have overviewed transcriptional regulation of storage root formation, proteomic regulation of storage root formation, ethylene regulation of storage root formation, auxin regulation of storage root formation, gene expression regulation of storage root formation, and metabolism regulation of storage root formation. We have reviewed the basic regulatory principles of storage root formation from the network of genomics to proteomics and metabolism in plants that will be valuable to research work in storage root growth and development regulation at the molecular level.

Keywords*— *Gene expression, post-transcriptional gene silencing, storage root development.

I. INTRODUCTION

Systems biology approaches are important and successful in understanding complex biological processes through molecular mechanisms involving the interaction of large numbers of genes. However, there are significant limitations in many of these methods (Slovak et al. 2016). For example, the mechanisms of the Si-mediated protection against metal deficiency remain poorly understood. Recently, it has been proposed that Si may act by an interaction with this biometal in the root apoplast contributing to its movement through the plant, mitigating Zn deficiency symptoms (Pascual et al. 2016). Plant adaptation to limited phosphate availability comprises a wide range of responses to internal phosphate sources and to enhance phosphate acquisition. In Arabidopsis, root growth modulation correlates with an altered expression of cell wall modifying enzymes and changes in the pectin network of the phosphate-deprived root tip, indicating that pectins are involved in iron binding and phosphate mobilization (Hoehenwarter et al. 2016). In sweet potato, storage roots develop from adventitious roots present in stem cuttings that serve as propagation material. Nodal position has a significant effect on the developmental status and number of root primordia inside the stem. Environmental conditions affect adventitious roots initiation, development, and capacity to form storage roots (Ma et al. 2015).

Turnips (*Brassica rapa* subsp. rapa) represent one of the morphotypes that form tubers and can be used to study the genetics underlying storage organ formation. The enlarged turnip tuber consists of both hypocotyl and root tissue, but the proportion of the two tissues differs between accessions. The ratio of sucrose to fructose and glucose differed among accessions. Vernalization resulted in reduced flowering time and smaller tubers for the Asian turnips (Zhang et al. 2014). The maintenance of the symbiotic characteristics of the incorporated bacterial strains was important in the formation of nodules in the soybean seedlings. A larger number of nodules formed in soybean seedlings from seeds inoculated with rhizobia demonstrated that there is a great alternative to the usual protector inoculants because of its unprecedented capacity to control the release of bacteria (Damasceno et al. 2013). Intraspecific variability in root colonization, extraradical growth pattern, and survival after cold storage of *Lactarius deliciosus* isolates was determined in pure culture conditions using *Pinus pinaster* as a host plant, indicating tolerance to cold water storage of *L. deliciosus* was isolate dependent (Parlade et al. 2011).

Physicochemical stability and biological activity of *Withania somnifera* root aqueous extract were affected by storage conditions. Temperature and humidity are important for storage conditions and shelf life of ashwagandha formulations (Patil et al. 2010). In response to suboptimal temperatures, plants increase root growth, build-up carbohydrates, and display typical morphological and anatomical changes. For carrot, suboptimal temperature promoted reserve structures, rather than the increase in carbohydrate concentration typical of most temperate annual species and woody perennials (Gonzalez et al. 2009). In this review, we overview transcriptional regulation of storage root formation, proteomic regulation of storage root formation, ethylene regulation of storage root formation, auxin regulation of storage root formation, gene expression regulation of storage root formation, metabolism regulation of storage root formation (Fig. 1). This review describes the

basic regulatory principles of storage root formation in plants and will be valuable to research work in storage root growth and development regulation at the molecular level.

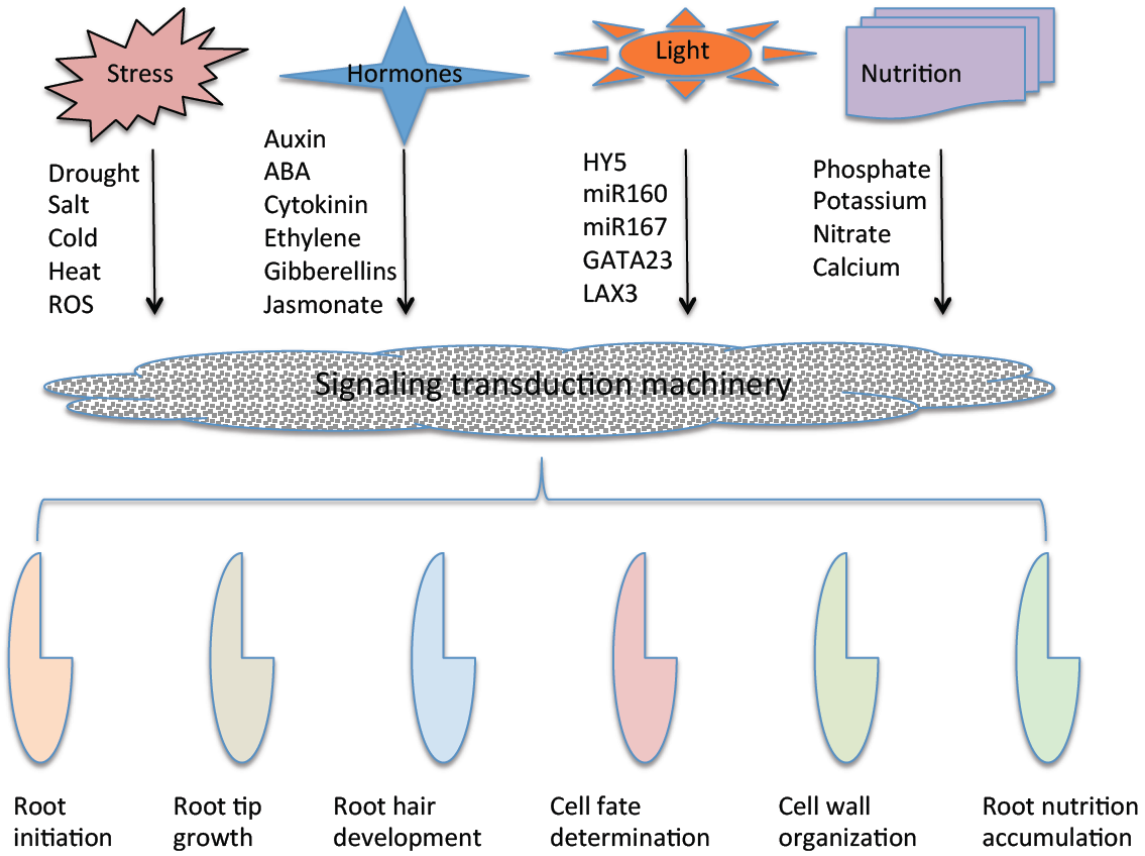

FIG. 1. OVERVIEW OF SIGNALING TRANSDUCTION MACHINERY RELATED TO STRESS, HORMONE, LIGHT, AND NUTRITION THAT ARE INVOLVED IN STORAGE ROOT INITIATION, GROWTH, DEVELOPMENT, AND STARCH AND PROTEIN ACCUMULATION IN PLANTS

II. DIFFERENT REGULATIONS OF STORAGE ROOT FORMATION

2.1 Transcriptional regulation of storage root formation

Sweet potato accumulates large quantities of starch in the storage roots and has been shown to give comparable or superior ethanol yields to corn per cultivated acre in the southeast. Thermostable and thermoactive amylolytic enzymes convert starch to fermentable sugars. The gene encoding a hyperthermophilic alpha-amylase from Thermotoga maritima was expressed in transgenic sweet potato. The transgene did not affect normal storage root formation and can facilitate cost effective starch conversion to fermentable sugars (Santa-Maria et al. 2011). Flavonoid 3'-hydroxylase is an important enzyme that determines the hydroxylation pattern of anthocyanins. Flavonoid 3'-hydroxylase was constitutively expressed in fibrous roots, thick roots, and storage roots. During storage root formation, Flavonoid 3'-hydroxylase was expressed most abundantly in the storage roots, suggesting that the anthocyanin biosynthesis is also active in the under-ground organs (Zhou et al. 2012).

Adventitious root (AR) formation in the stem base (SB) of cuttings is the basis for propagation of many plant species. Analyses of phytohormone-related genes disclosed multifaceted changes of the auxin transport system, auxin conjugation and the auxin signal perception machinery indicating a reduction in auxin sensitivity and phase-specific responses of particular auxin-regulated genes. Genes involved in ethylene biosynthesis and action showed a more uniform pattern as a high number of respective genes were generally induced during the whole process of AR formation. The important role of ethylene for stimulating AR formation was demonstrated by the application of inhibitors of ethylene biosynthesis and perception as well as of the precursor aminocyclopropane-1-carboxylic acid, all changing the number and length of AR. A model is proposed showing the putative role of polar auxin transport and resulting auxin accumulation in initiation of subsequent changes in auxin homeostasis and signal perception with a particular role of Aux/IAA expression. These changes might in turn guide the entrance into the different phases of AR formation. Ethylene biosynthesis, which is stimulated by

wounding and does probably also respond to other stresses and auxin, acts as important stimulator of AR formation probably via the expression of ethylene responsive transcription factor genes, whereas the timing of different phases seems to be controlled by auxin (Druege et al. 2014).

Radish (*Raphanus sativus* L.) is a widespread agricultural plant forming storage root due to extensive secondary growth that involves cambium proliferation and differentiation of secondary conductive tissues. CLE peptides are a group of peptide phytohormones which play important role in the regulation of primary meristems such as SAM, RAM, and procambium, as well as secondary meristems. In radish, 18 CLE genes of radish (RsCLEs) have been identified and their expression at different stages of root development in *R. sativus* has been measured. The results demonstrated that RsCLE2, 19 and 41 genes play roles in the development of storage root of radish. RsCLE19 plays a role in auxin-dependent processes of xylem differentiation and RsCLE41 stimulates cambium activity (Gancheva et al. 2016).

The storage roots of *Callerya speciosa* that are derived from fibrous roots are used medicinally. To detect key genes involved in storage roots formation, Illumina sequencing of the C. speciosa storage roots and fibrous roots has been applied. After expression profiling, 4538 differentially expressed genes were identified. The KEGG pathway enrichment analysis revealed changes in the biosynthesis of cytokinin, phenylpropanoid, starch, sucrose, flavone and other secondary metabolites. Transcription factor-related differentially expressed genes (DEGs) were also identified, including such gene families as GRAS, COL, MIKC, ERF, LBD, and NAC. The DEGs related to light signaling, starch, sugar, photohormones and cell wall-loosening might be involved in the formation of storage roots. This study provides the first transcriptome profiling of C. speciosa roots, data that will facilitate future research of root development and metabolites with medicinal value as well as the breeding of C. speciosa (Xu et al. 2016a).

2.2 Proteomic regulation of storage root formation

Metabolism and regulatory processes in leaf may have an impact on tuber formation. To search for leaf proteins putatively involved in regulating tuber generation and development in cassava (*Manihot esculenta* Crantz), comparative proteomic approaches have been applied to monitor differentially expressed leaf proteins during root transition from fibrous to tuberous. A number of protein spots whose abundance were significantly altered were successfully identified by ion trap LC-MS/MS during growth. The proteins span various functional categories from antioxidant and defense, carbohydrate metabolism, cyanogenesis, energy metabolism, miscellaneous and unknown proteins, suggesting possible metabolic switches in the leaf that may trigger/regulate storage root initiation and growth. The results can help understand how biochemical processes in cassava leaves may be involved in storage root development (Mitprasat et al. 2011). The symbiosome membrane represents a specialized plant membrane that forms both a structural and a functional interface between the legume plant and its bacterial counterpart. Symbiosome membrane protein profile from the model system Medicago truncatula and the corresponding bacterium Sinorhizobium meliloti was examined using two-dimensional electrophoresis and microcapillary high-performance liquid chromatography (HPLC) tandem mass spectrometry. The identities of 51 proteins were obtained and these proteins were categorized into functional classes to indicate biochemical roles. Symbiosome membrane proteins include an H(+)-ATPase, ENOD16, ENOD8, nodulin-25, BiP, HSP70, PDI, multifunctional aquaporin, a putative syntaxin, and other proteins of known and unknown identity and function. The majority of the proteins identified were involved with protein destination and storage. These results allow us to understand better the biochemical composition of the symbiosome membrane and thus provide a basis to hypothesize mechanisms of symbiosome membrane formation and function (Catalano et al. 2004).

Different proteomic approaches, including SDS-PAGE, classical two-dimensional gel electrophoresis (2DE), high-resolution 2DE, mass spectrometry, and iTRAQ-based analysis, have contributed for characterization of proteome in plants. A large number of proteins have been identified, including those involved in the storage root formation and post-harvest physiological deterioration processes (Batista de Souza et al. 2015). In sweet potato (*Ipomoea batatas* (L.) Lam.), a proteomic analysis was conducted on the pencil and storage roots of the light orange-fleshed sweet potato cultivar, Yulmi, to understand protein function in root development. Two-dimensional gel electrophoresis showed that expression of 30 protein spots differed between pencil and storage roots: 15 proteins were up-regulated or expressed in pencil roots and 15 in storage roots. Binding protein isoform A, catechol oxidase, peroxidases, ascorbate peroxidase, endochitinase, flavanone 3-hydroxylase, protein disulfide isomerase, anionic peroxidase, putative ripening protein, sporamin B, sporamin A, and sporamin A precursor are play roles in storage root formation. These results provide important insight into sweet potato proteomics and indicate that the reduction of carbon flow toward phenylpropanoid biosynthesis and its delivery to carbohydrate metabolism is a major event in storage root formation (Lee et al. 2015).

Comparison of the expression profiles of storage root proteome at various developmental stages was investigated in cassava using two-dimensional gel electrophoresis and LC-MS/MS. Experimental results demonstrated that the secondary growth was confirmed to be essential during the development of cassava storage root and 8 functional groups including protein folding and degradation, energy, metabolism, secondary metabolism, stress response, transport facilitation, cytoskeleton, and unclassified function have been identified. The expression profiling of membrane proteins revealed the proteins involved in protein folding and degradation, energy, and cell structure were highly expressed during early stages of development (Naconsie et al. 2016). To sustain root function in the hypoxic environment, a key adaptation for waterlogging tolerant plants is the formation of adventitious roots. To understand the molecular mechanisms underlying root emergence, the iTRAQ-based quantitative proteomics approach was employed to map the proteomes of hypocotyls cells under control and waterlogging conditions. A total of 5508 proteins were identified and 146 were differentially regulated proteins, of which 47 and 56 DRPs were specific to tolerant and sensitive line, respectively. Alcohol dehydrogenases (ADH), 1-aminocyclopropane-1-carboxylicacid oxidases, peroxidases, 60S ribosomal proteins, GSDL esterases/lipases, histone deacetylases, and histone H5 and were strongly overrepresented to manage the energy crisis, promote ethylene release, minimize oxidative damage, mobilize storage lipids, and stimulate cell division, differentiation and growth. These findings provided valuable information for the breeding of plants with enhanced tolerance to waterlogging (Xu et al. 2016b).

2.3 Ethylene regulation of storage root formation

Ethylene may affect rooting responses and storage root formation. In etiolated mung-bean seedlings treated with the auxins alpha-naphthaleneacetic acid, gamma-(indole-3)-n-butyric acid (IBA) and 2,4,5-trichloro-phenoxypropionic acid, no relationship between the abilities of the auxins to induce root formation and their capacities for inducing ethylene production. Exposure of cuttings to ethylene or (2-chloroethyl) phosphonic acid (Ethephon), hypobaric storage of treated cuttings, and exposure of auxin-treated cuttings to 7% CO_2 also indicated that ethylene is not directly involved in initiation of adventitious roots in this plant material (Batten and Mullins 1978). In *Brassica rapa* root, high pressure treated samples formed unique green-blue color during 7-d storage at 4 degrees C, indicating that the mechanism of green-blue compound formation would be from biochemical pathway involved in enzymatic reactions with ethylene (Ueno et al. 2009).

Genes involved in ethylene biosynthesis and action showed a more uniform pattern as a high number of respective genes were generally induced during the whole process of root formation. The important role of ethylene for stimulating root formation was demonstrated by the application of inhibitors of ethylene biosynthesis and perception as well as of the precursor aminocyclopropane-1-carboxylic acid. A model is proposed showing the putative Ethylene biosynthesis, which is stimulated by wounding and does probably also respond to other stresses and auxin, acts as important stimulator of root formation probably via the expression of ethylene responsive transcription factor genes, whereas the timing of different phases seems to be controlled by auxin (Druege et al. 2014).

In, *R. glutinosa* tuberous, root formation is involve in different developmental stages including seeding, elongation, pre-expanding, mid-expanding, late-expanding and maturity stage. The anatomic characteristics indicated that the fission of secondary cambium initiated the tuberous root expansion, and the continuous and rapid division of secondary cambium and accessory cambium kept the sustained and rapid expansion of tuberous root. The quantitative analysis suggested that the genes related to biosynthesis and response of the IAA, CK, ABA,ethylene, JA and EB were up-regulated expressed, meanwhile, GA synthesis and response genes were down-regulated expressed and the genes of GA negative regulation factors were up-regulated expressed. The maximum levels of most genes expression occurred in the elongation and pre-expansion stage, indicating these two stages were the key periods to the formation and development of tuberous roots (Wang et al. 2014). Waterlogging is a common abiotic stress and cucumber waterlogging tolerant line Zaoer-N seedlings adapt to waterlogging stress by developing a larger number of roots in hypocotyls. Increased ethylene release may protect root from stress damage by mobilizing storage lipids and stimulating cell division, differentiation and growth (Xu et al. 2016b).

2.4 Auxin regulation of storage root formation

Auxins induce root formation and studies with mixtures of 3-indoleacetic acid and IBA indicated that auxins were directly involved in initiation of adventitious roots in plants (Batten and Mullins 1978). In carrot (Daucus carota, L.), treatment of unwounded carrot storage roots with 10 microM 2,4-dichlorophenoxy-acetic acid, indoleacetic acid, or naphthalene-1-acetic acid resulted in the accumulation of DcPRP1 transcripts to a level equal to that seen in wounded tissue (Ebener et al. 1993). In walnut, a short auxin treatment suppressed the formation of large roots and induced numerous tiny rootlets dispersed all over the surface of the cotyledons (Gutmann et al. 1996). Plants use several mechanisms to regulate levels of the auxin

indole-3-acetic acid (IAA), including the formation and hydrolysis of amide-linked conjugates that act as storage or inactivation forms of the hormone. To examine the in vivo importance of auxin-conjugate hydrolysis, a triple hydrolase mutant, ilr1 iar3 ill2, which is deficient in three of these hydrolases has been generated. The hydrolase mutant phenotypic profiles on different conjugates reveal the in vivo activities and relative importance of ILR1, IAR3, and ILL2 in IAA-conjugate hydrolysis and that amidohydrolases contribute free IAA to the auxin pool during germination in Arabidopsis (Rampey et al. 2004).

The effects of cytokinins and auxins on the formation of storage roots in vitro were examined in cassava. Auxin and cytokinin supplementation were absolutely required for in vitro storage root regeneration; these roots were not able to develop secondary growth, but formed a tissue competent for starch storing (Medina et al. 2007). In sweet potato (Ipomoea batatas cv. 'Jinhongmi'), MADS-box protein cDNA (SRD1) has been isolated from an early stage storage root cDNA library. Transcripts of SRD1 were detected only in root tissues, with the fibrous root having low levels of the transcript and the young storage root showing relatively higher transcript levels. SRD1 mRNA was mainly found in the actively dividing cells, including the vascular and cambium cells of the young storage root, indicating that SRD1 plays a role in the formation of storage roots by activating the proliferation of cambium and metaxylem cells to induce the initial thickening growth of storage roots in an auxin-dependent manner (Noh et al. 2010).

Very-long-chain fatty acids (VLCFAs) are essential for many aspects of plant development and necessary for root formation. Identification of the acetyl-CoA carboxylase PASTICCINO3 and the 3-hydroxy acyl-CoA dehydratase PASTICCINO2 revealed that VLCFAs are important for cell proliferation and tissue patterning (Roudier et al. 2010). Polar auxin transport and resulting auxin accumulation in initiation of subsequent changes in auxin homeostasis and signal perception with a particular role of Aux/IAA expression might guide the entrance into the different phases of root formation. Auxin, acts as important stimulator of root formation probably via the expression of ethylene responsive transcription factor genes (Druege et al. 2014). In *Raphanus sativus*, storage root formation is due to extensive secondary growth which involves cambium proliferation and differentiation of secondary conductive tissues and auxins play important role in the regulation of primary meristems such as SAM, RAM, and procambium, as well as secondary meristems (Gancheva et al. 2016).

2.5 Gene expression regulation of storage root formation

In carrot (*Daucus carota* L.), genomic clone (DcPRP1) was isolated on the basis of its homology to previously described cDNAs encoding a wound-inducible, proline-rich cell wall protein. Expression of DcPRP1 is developmentally regulated and linked to the formation of storage roots, where this gene is expressed at high levels after wounding (Ebener et al. 1993). In sweet potato (Ipomoea batatas), storage root coincides with starch accumulation made using cleaved products of imported photoassimilate sucrose. Sucrose synthase (SuSy) was found to be significantly more frequent in storage root than in fibrous root. SuSy was the most abundant carbohydrate-metabolism gene in the storage-root. SuSy was the most actively expressed enzyme in sucrose metabolism in developing storage root and was predominant for sucrose cleavage related to starch-accumulation (Li and Zhang 2003). In Cassava (Manihot esculenta Crantz) storage roots, organs accumulating large amounts of starch, develop from primary roots via secondary growth (Zhang et al. 2003). Cassava storage roots result from swelling of adventitious roots by secondary growth. Comparative gene expression study in adventitious and storage roots in order to identify genes possibly related to storage organ formation revealed five genes with higher expression levels in secondary xylem of storage roots than adventitious roots. Among them, the Mec1 gene coding for Pt2L4 glutamic acid-rich protein and a putative RING Zinc Finger and LEA protein genes were strongly induced in secondary xylem tissue (de Souza et al. 2004). Auxins are hormones important for numerous processes throughout plant growth and development. Formation and hydrolysis of amide-linked conjugates act as storage forms of the hormone that contribute free IAA to the auxin pool during germination in Arabidopsis (Rampey et al. 2004).

Differential display analysis has been used to identify the genes involved in storage root formation. In sweet potato, the expression of SRF1, SRF2, SRF3, SRF5, SRF6, SRF7, and SRF9 increased during storage root formation, whereas the expression of SRF4, SRF8, and SRF10 decreased (Tanaka et al. 2005). In potato, radish, turnip, and ginger, lyophilization can be used to preserve RNA in high starch- and phenolic-containing plant tissues for studies on gene expression (Kumar et al. 2007). Using this method, MADS-box protein cDNA (SRD1) has been isolated from an early stage storage root cDNA library in sweet potato. SRD1 plays a role in the formation of storage roots by activating the proliferation of cambium and metaxylem cells to induce the initial thickening growth of storage roots in an auxin-dependent manner (Noh et al. 2010). In Cassava, gene expressions during storage root development provide important information on storage root formation and starch. Calcium-dependent protein kinase (CDPK) (MeKD83), entkaurene synthase (KS) (MeKD106) and hexose transporter

(HT) (MeKD154) showed root-specific expression patterns. KS and HT may involve in transient induction of CDPK expression, which may play an important role in the signaling pathway of storage root initiation. Sulfite reductase may involve in storage root development by facilitating sulfur-containing protein biosynthesis (Sojikul et al. 2010). In poplar, PHOTOPERIOD RESPONSE 1 (PHOR1) is most highly expressed in roots and induced by short days, while PtPHOR1_2 is more uniformly expressed throughout plant tissues and is not responsive to short days. PtPHOR1_1 effects were restricted to roots while PtPHOR1_2 had similar effects on aerial and below-ground development. The effect of PHOR1 suppression on starch accumulation was coupled with growth-inhibiting effects in both roots and shoots, suggesting that PHOR1 is part of a mechanism that regulates the allocation of carbohydrate to growth or storage in poplar (Zawaski et al. 2012).

To identify the molecular mechanisms involved in the initiation of storage root formation, by performing a detailed transcriptomic analysis of initiating storage roots using next-generation sequencing platforms. The differential expression profiles indicated down-regulation of classical root functions, such as transport, as well as down-regulation of lignin biosynthesis in initiating storage roots, and up-regulation of carbohydrate metabolism and starch biosynthesis. Carbohydrate metabolism and starch biosynthesis, are major events involved in storage root initiation (Firon et al. 2013). The role of an expansin gene (IbEXP1) in the formation of the storage root (SR) was investigated by expression pattern analysis and characterization of IbEXP1-antisense sweet potato. The transcript level of IbEXP1 was high in the fibrous root (FR) and petiole at the FR stage, but decreased significantly at the young storage root (YSR) stage. IbEXP1 plays a negative role in the formation of SR by suppressing the proliferation of metaxylem and cambium cells to inhibit the initial thickening growth of SRs (Noh et al. 2013). Forskolin, a complex labdane diterpenoid found in the root of Coleus forskohlii (Lamiaceae), has received attention for its broad range of pharmacological activities, yet the biosynthesis has not been elucidated. Expression profiling and phylogenetic analysis of the CfTPS family further support the functional diversification and distinct roles of the individual diterpene synthases and the involvement of CfTPS1 to CfTPS4 in specialized metabolism and of CfTPS14 and CfTPS15 in general metabolism. Our findings pave the way toward the discovery of the remaining components of the pathway to forskolin, likely localized in this specialized cell type, and support a role of oil bodies as storage organelles for lipophilic bioactive metabolites (Pateraki et al. 2014). Several cassava proteins have been identified, including those involved in the storage root formation and post-harvest physiological deterioration processes (Batista de Souza et al. 2015).

Development of storage roots is a process associated with a phase change from cell division and elongation to radial growth and accumulation of massive amounts of reserve substances such as starch. In cassava storage root development, MeAGL20 is a factor that might play an important role at the onset of storage root initiation (Sojikul et al. 2015). The peptides play a role during storage root formation. The expression level of RsCLE19 strongly decrease in response to exogenous cytokinin and expression level of RsCLE41 strongly decrease in response to exogenous auxin, suggesting that RsCLE19 may play a role in auxin-dependent processes of xylem differentiation and RsCLE41 stimulates cambium activity (Gancheva et al. 2016). In petunia, dark exposure before planting enhances the carbon sink competitiveness of the rooting zone and that expression and activity of invertases contribute to the shift in carbon allocation (Klopotek et al. 2016). In Cassava, The proteins with differential expression pattern were analysed and identified to be associated with stress response. The expression profiling of membrane proteins revealed the proteins involved in protein folding were highly expressed during early stages of development. Possible role of identified proteins were discussed in relation with the activities during storage root maturation in cassava (Naconsie et al. 2016). Nitric oxide (NO) is a signaling molecule that plays important role in development of plant. In soybean, expression of peroxidase (POX), catalase (CAT), vegetative storage protein (VSP), and nitrite reductase (NR) genes were up-regulated and high affinity K+ transporter (HKT1), lipoxygenase (LOX), polyphenol oxidase (PPO), and pyrroline-5-carboxylate synthase (P5CS) genes were down-regulated during root development and growth under stress, through NO signaling (Vaishnav et al. 2016).

2.6 Metabolism regulation of storage root formation

Phospholipase D in plant storage tissues and seeds may be related to the rapid growth involved in their formation rather than being necessary for the utilization of their food reserve substances (Quarles and Dawson 1969). In tobacco (*Nicotiana tabacum* L.), protein bodies contain heme protein with strong oxidase activity may convey a specific function to proteinoplasts (Vigil and Ruddat 1985). Sucrose plays a central role with respect to both short-term storage and distribution of photoassimilates formed in the leaf. Sucrose is synthesized in the cytosol, transiently stored in the vacuole and exported via the apoplast. Expression of cytosolic yeast invertase resulted in the accumulation of starch and soluble sugars in plants (Sonnewald et al. 1991). The neutral lipids of *G. intraradices* increased continuously in the intraradical mycelium, while vesicle occurrence decreased after initial rapid root colonization by the fungus. S. *calospora* does not form vesicles and

accumulated more neutral lipids in extraradical than in intraradical mycelium, suggesting that the ratio of neutral lipids to phospholipids is more important than is the presence of vesicles in determining the storage status of AM fungi (van Aarle and Olsson 2003).

The role of nitrogen- and storage-affected carbohydrate availability in rooting of pelargonium cuttings has been determined. The results indicate that adventitious rooting of pelargonium cuttings can be limited by the initial amount of nitrogen reserves. However, this relationship reveals only small plasticity and is superimposed by a predominant effect of carbohydrate availability that depends on the initial leaf sugar levels, when high-light adaptation and low current light conditions impair net carbon assimilation (Druege et al. 2004). The occurrence of oligosaccharide motifs of pectic polysaccharides are spatially regulated in sugar beet root cell walls and that the spatial patterns vary between cell types suggesting that structural variants of pectic polymers are involved in the modulation of cell wall properties (Guillemin et al. 2005). The roots of *Epipremnum aureum* take up exogenously fed nicotine as a xenobiotic and provide insight into the mechanisms of uptake, transport and storage of nicotine as a xenobiotic (Weidner et al. 2005). The starch pool in non-needle parts, which can be used for xylem formation, drew approx. 43% of its carbon from the previous year's photoassimilate, indicating that carbon storage is a key mechanism (Kagawa et al. 2006).

It was discovered that development of P-toxicity symptoms in H. prostrata is related to its low capacity to down-regulate net P-uptake rates. In response to higher P supply, G. crithmifolia does not develop symptoms of P toxicity (Shane and Lambers 2006).

Protein storage and lytic vacuoles in root tips of barley (Hordeum vulgare) and pea (Pisum sativum) seedlings were initially separate compartments that later fused to form a central vacuole during cell elongation (Olbrich et al. 2007). Correlation of increased accumulation of both ferritin polypeptide and mRNA with actual in situ localization of ferritin allowed ferritin synthesis in the developing, indeterminate-type root nodules to be related to differentiating bacteroid tissue (Strozycki et al. 2007). Mycorrhiza formation is the consequence of a mutualistic interaction between certain soil fungi and plant roots that helps to overcome nutritional limitations faced by the respective partners (Nehls 2008).

The mechanisms for the detoxification of xenobiotics in plants are closely related to the mammalian system. With LC-MS technique, paracetamol and its metabolites in root cells of *A. rusticana* were identified (Huber et al. 2009). In Brassica rapa root, high pressure treated samples formed unique green-blue color, indicating that green-blue compound formation would be based on biochemical pathway for a unique green-blue pigment synthesis, containing O2-dependent steps and possibly enzymatic reactions (Ueno et al. 2009). Very-long-chain fatty acids (VLCFAs) are essential for plant development and for the synthesis of seed storage triacylglycerols, epicuticular waxes, and sphingolipids. Exogenous VLCFAs rescue lateral root organogenesis and polar auxin distribution, indicating their direct involvement during plant development (Roudier et al. 2010). In cassava, competition of photoassimilate partitioning between the shoot and the root organs, has an impact on storage root development (Mitprasat et al. 2011). In sweet potato, starch conversion to fermentable sugars is carried out at high temperatures and requires the action of thermostable and thermoactive amylolytic enzymes (Santa-Maria et al. 2011). In poplar, PHOTOPERIOD RESPONSE 1 (PHOR1) suppression led to increased starch accumulation in both roots and stems, suggesting PHOR1 is part of a mechanism that regulates the allocation of carbohydrate to growth or storage (Zawaski et al. 2012).

To identify the molecular mechanisms involved in the initiation of storage root formation, transcriptomic analysis of initiating storage roots using next-generation sequencing platforms has been performed. The results indicate genes that are involved in the earliest stage of storage root formation, highlighting the reduction in carbon flow toward phenylpropanoid biosynthesis and its delivery into carbohydrate metabolism and starch biosynthesis, as major events involved in storage root initiation are related to storage root initiation identified (Firon et al. 2013). Reduced vacuolar invertase activity leads to reduced net photosynthesis in the shoot and lowered root respiration, and affords an increased root/shoot ratio and roots have very capacity for carbon storage for their maintenance metabolism (Brauner et al. 2014). Carbon and nitrogen remobilization/storage processes are key to tree growth and survival. The pattern of carbon compound accumulation in branches supports the hypothesis of a preferential allocation of carbon towards growth until the end of wood formation in juvenile trees, at the expense of the replenishment of carbon stores, while mature trees start allocating carbon to storage right after budburst (Gilson et al. 2014). Vacuole formation from provacuoles was observed in cells newly produced by root meristem. Decreased expression of TIP3s was associated to the transformation of protein storage vacuoles to vacuoles, whereas enhanced expression of a TIP2 homologue was closely linked to the fast cell elongation (Novikova et al. 2014). In *C. forskohlii*, expression profiling and phylogenetic analysis of the diterpene synthase candidates (CfTPSs) family support

the functional diversification and distinct roles of the individual diterpene synthases and the involvement of CfTPS1 to CfTPS4 in specialized metabolism and of CfTPS14 and CfTPS15 in general metabolism (Pateraki et al. 2014). It has been suggested that these nitrogen-based secondary metabolites act as storage reserves of nitrogen. In sorghum, three key genes, CYP79A1, CYP71E1 and UGT85B1, are essential for synthesis of the cyanogenic glucoside dhurrin (Blomstedt et al. 2016).

The formation of storage organs is a central part of the life cycle of an arbuscular mycorrhizal fungus (AMF). The AMF's extraradical mycelium produces its storage organs within dead roots in preference to air space in the substrate. Dead roots may indirectly supply nutrients to AMF (Muller et al. 2016). Mycorrhiza formation is the consequence of a mutualistic interaction between certain soil fungi and plant roots that helps to overcome nutritional limitations faced by the respective partners. Current knowledge on fungal strategies to obtain carbohydrates from its host and plant strategies to enable, but also to control and restrict (under certain conditions), carbon transfer are summarized (Nehls 2008). The development of intraradical and extraradical mycelia of the arbuscular mycorrhizal (AM) fungi Scutellospora calospora and Glomus intraradices when colonizing Plantago lanceolata was compared with the amounts of signature fatty acids (van Aarle and Olsson 2003). Analysis of symbiotic genes showed that the nifH gene was only detected for the Klebsiella-like isolates and the nodC gene could not be amplified by PCR or be detected by Southern blotting in any of the isolates. The results obtained support the idea that these isolates are opportunistic bacteria able to colonize nodules induced by rhizobia (Ibanez et al. 2009).

Post-transcriptional processing of primary transcripts can significantly affect the corresponding protein products. In wheat (Triticum aestivum), TaRSZ22, TaSRp30, TaU1-70K, and the large and small subunits of TaU2AF, are wheat homologues of known plant splicing factors that are mainly expressed in roots (Lopato et al. 2006).

Increasing the sucrose concentration in the calcium chloride polymerisation medium significantly reduced regrowth from encapsulated nodal cuttings of accession TME 60444. The high frequency of plant regrowth from alginate-coated micropropagules coupled with high viability percentage after 28 days of storage is highly encouraging for the exchange of cassava genetic resources (Danso and Ford-Lloyd 2003). In cassava, storage-root formation is initiated when plants are 1 to 2 months old. The production loss caused by M. incognita to young SS4 plants was due to a reduction of storage-root number rather than a reduction in individual storage-root weight (Makumbi-Kidza et al. 2000). In carrot, root produced invertase resulted in a slower rate of enzyme production and a lower final level (Bradshaw et al. 1970). In red beet (Beta vulgaris L.), Invertase activity in the disks has been measured by a polarimetric method. Trisaccharides that appear in sugar-beet disks during the washing process have been isolated and identified; their formation also suggests that a higher-plant invertase is acting. in relation to protein synthesis in washed storage-tissue slices, and the occurrence of high invertase activity in growing plant cells (Bacon et al. 1965). Understanding metabolism regulation of storage root formation and their molecular mechanisms in plants may have practical application in agricultural production, environmental protection, and plant molecular breeding.

III. CONCLUSION

Molecular mechanisms regulating storage root initiation and formation are important for the growth and development in plants because storage roots provide nutrients, water, disease resistance, and energy storage. In this review, we have overviewed transcriptional regulation, proteomic regulation, ethylene regulation, auxin regulation, gene expression regulation, and metabolism regulation of storage root formation. We have reviewed the basic regulatory principles of storage root formation from the network of genomics to proteomics and metabolism in root formation at the cellular and molecular level, as well as the interactions of genes and gene networks. Understanding molecular mechanisms regulating storage root formation in plants may have practical application in plant molecular breeding.

AUTHORS' CONTRIBUTIONS

WT wrote the manuscript. All authors participated, contributed to design of research, performed, and evaluated the experiments. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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