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REVIEW

Recent Progress in Molecular Studies on Storage Root Formation in Sweetpotato (*Ipomoea batatas*)

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Abstract

Elucidating the mechanisms underlying the formation of storage roots is important for further improvement of sweetpotato. Although the process of storage root formation in sweetpotato has been extensively studied anatomically and physiologically, the genetic and molecular mechanisms remain largely unknown. Recently, extensive gene expression analysis has identified numerous genes differentially expressed between fibrous and storage roots. The results of these analyses agreed with previous anatomical and physiological observations in that genes related to starch and storage protein biosynthesis were up-regulated, and those related to lignin biosynthesis were down-regulated in the storage roots. Also, molecular biological studies and analyses using transgenic sweetpotato plants have suggested that an expansin protein and several transcription factor genes, such as a Dof-type zinc finger protein, MADS-box proteins, and KNOXI proteins, are involved in the characteristic physiological process of storage root formation. Furthermore, ongoing whole-genome sequencing of sweetpotato and the related *Ipomoea* species is expected to identify genetic differences related to storage root formation. Although these studies are valuable as a first step in elucidating the critical genes and genetic network that control the formation of storage roots, further physiological, genetic, and molecular biological studies are needed to reveal the entire molecular process.

Discipline: Biotechnology
Additional key words: tuberous root, development, gene

Introduction

Sweetpotato (*Ipomoea batatas*) is an important food crop with global production totaling about 100 million tonnes (FAO 2013), and shows the highest daily energy yield per hectare among the major food crops available in developing countries (Woolfe 1992). Although sweetpotato originated in the tropical Americas, about 80% of its worldwide production now comes from Asian countries. Rich in vitamins and minerals, and also containing a large amount of starch, the storage roots of sweetpotato are an important source of nutrition, especially in developing countries (Woolfe 1992). In addition to table use, sweetpotato is utilized as an important raw material for food processing and industrial purposes, such as starch and pigment production. Given its outstanding calorie productivity and relatively high tolerance to unfavorable weather and cultivation conditions, sweetpotato is expected to become a candidate crop for feeding the world's growing population, but its world average yield peaked in the 1990s and has not increased since (FAO 2013). To overcome the yield plateau for this important crop, the physiological and genetic basis of sweetpotato productivity should be clarified.

The storage roots of sweetpotato are formed by a secondary thickening of adventitious roots formed on the underground nodes of transplanted stem cuttings. These storage roots accumulate photosynthetic products as a sink organ throughout the cultivation period of sweetpotato. This long duration of dry matter being accumulated in the storage roots is a major reason for the high and stable productivity of sweetpotato (Tsuno and Fujise 1965). Grafting experiments using various sweetpotato cultivars and wild-relative plants (*I. trifida*) have also suggested that the dry matter productivity of sweetpotato is highly dependent on the sink potential of developing storage roots (Hozyo & Park 1971, Hahn 1977, Nakatani et al. 1988). Furthermore, Kokubu (1973) suggested that the level of starch accumulated in storage roots is related to their anatomical structure.

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Thus, elucidating the mechanism of storage root formation is essential for understanding sweetpotato productivity.

As described below, the anatomical and physiological processes of storage root formation in sweetpotato have been well described, although its genetic and molecular basis remains largely unknown. This is mainly attributed to the difficulties posed in a genetic analysis of sweetpotato, due to its autohexaploid ($2n = 6x = 90$) and allogamous nature. Recently, technical advances in gene expression analysis and the construction of transgenic sweetpotato plants have enabled a molecular biological approach to identifying the genes involved in storage root formation. The aim of this review is to summarize the recent progress made in molecular studies on the formation of storage roots in sweetpotato, as well as to provide a brief summary of past anatomical, physiological, and genetic studies, and discuss future prospects in this research area.

Anatomical, physiological and genetic studies on storage root formation

The anatomical process of storage root formation has been well described in classical studies (McCormick 1916, Togari 1950, Wilson & Lowe 1973, Kokubu 1973). At the beginning of storage root development, vascular cambiums are formed between the protoxylem and protophloem of young fibrous roots, and become continuous to form a circular primary cambium. Subsequently, secondary meristems, including meristems surrounding vessels and anomalous secondary cambiums, differentiate in the xylem. Cell divisions and expansions in the primary cambium and secondary meristems, together with divisions of large parenchyma cells in the xylem, lead to an expansion of root diameter (Fig. 1). Togari (1950) suggested that higher activity of the primary cambium and a lower degree of lignification in the stele were necessary for the differentiation of young adventitious roots into storage roots. Kokubu (1973) analyzed the relationship between the anatomical characteristics and starch content of storage roots, and found that cultivars with a higher starch content show higher cell division activity at the primary and secondary cambiums.

In addition to these anatomical studies, physiological factors affecting the formation and thickening of storage roots, such as soil temperature, humidity, and nutrition, have been identified (Firon et al. 2009, Villordon et al. 2014). In addition, on-line systems to monitor the thickening of storage roots have also been reported (Eguchi et al. 1997, Villordon & LaBonte 2008) and used to analyze the optimum physiological conditions of storage root formation (Eguchi et al. 1998, 2003). Such systems may be effective for molecular studies, such as a close examination of gene expression during the formation of storage roots.

The involvement of endogenous plant hormones has also been suggested from physiological studies. The endogenous cytokinin level has been shown to rapidly increase in sweetpotato roots at the beginning of storage root formation (Nakatani & Komeichi 1991), whereas in a mutant with a late-storage, root-forming phenotype, this rapid increase was suppressed (Nakatani et al. 2002). The enhancement of storage root formation by exogenous application of a synthetic cytokinin has also been reported (McDavid & Alamu 1980). As a recent study in Arabidopsis showed

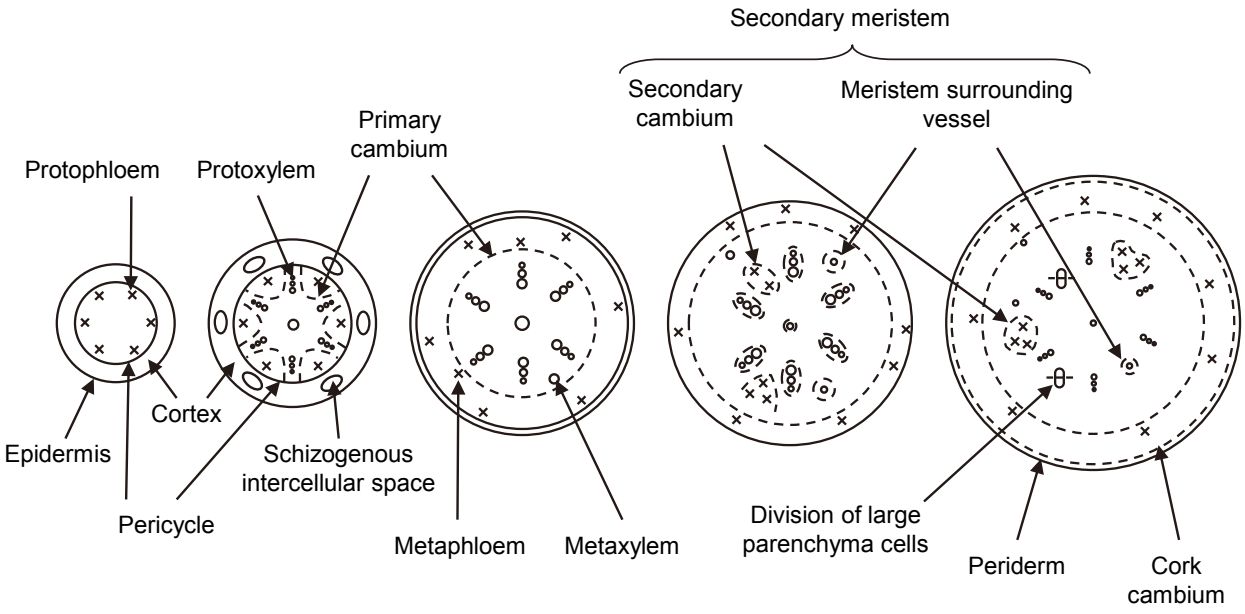

Fig. 1. Storage root formation of sweetpotato (modified from Kokubu 1973). Cross sections of adventitious roots in various stages of storage root formation (left to right) are shown schematically.

that cytokinins are central regulators of cambial activity (Matsumoto-Kitano et al. 2008), cytokinins probably play important roles in storage root formation through regulation of the primary cambium activity.

In contrast to the anatomical and physiological studies, only a few genetic studies have been conducted on storage root formation. Given the allogamous nature of sweetpotato, an analysis of F_1 populations by using the pseudo-testcross method (Grattapaglia & Sederoff 1994) is effective. However, a genetic analysis of autohexaploids is still difficult due to complicated segregation patterns. Shiotani (2006) analyzed F_1 progenies between diploid *I. trifida* lines with different root thickening capabilities, and suggested that the formation of storage root-like thickened roots was determined by a single recessive gene. Recently, genetic linkage maps of sweetpotato have been developed using DNA markers (Cervantes-Flores et al. 2008, Zhao et al. 2013), and quantitative trait loci (QTL) related to storage root yield have been reported (Cervantes-Flores et al. 2006, Chang et al. 2009, Li et al. 2014). Unfortunately, the number of markers used in these studies is insufficient to evenly cover the 90 chromosomes of sweetpotato. And because different marker sets were used in these investigations, it is difficult to compare the QTLs between those sets. In addition, as Li et al. (2014) pointed out, the number and location of yield-related QTLs are affected by environmental conditions, which could be a major obstacle for the fine mapping of these QTLs. For these reasons, it remains somewhat difficult to identify genes involved in the formation or thickening of storage roots from these yield-related QTLs.

To date, two of the best characterized sweet potato genes have been the sporamin and β-amylase genes, which encode the major storage proteins of sweetpotato storage roots.

Analysis of gene expression change during storage root formation

In order to identify candidates for key regulatory genes that initiate storage root formation and better understand the entire molecular process of storage root development, several studies have attempted gene expression analyses using adventitious roots in different developmental stages, and thus successfully identified genes showing differential expression between fibrous roots and storage roots (Table 1).

You et al. (2003) sequenced 2,859 clones of storage root cDNA and identified 39 genes putatively involved in gene regulation, signal transduction, and development. Of these 39 genes, 22 genes were shown to be differentially expressed between fibrous and storage roots. Tanaka et al. (2005) compared gene expression between fibrous, thick and storage roots of sweetpotato by using a simplified differential display method, and identified ten genes (named *SRF1* to *SRF10*) that are differentially expressed during the formation of storage roots. These genes included those involved in various physiological processes, such as sugar metabolism, signal transduction, and carotenoid biosynthesis. McGregor et al. (2005) and McGregor (2006) analyzed

Table 1. Identification of genes differentially expressed between fibrous and storage roots

Author (Year)	Analytical method	No. of differentially expressed genes identified	Functional characteristics of the differentially expressed genes
You et al. (2003)	cDNA sequencing and northern blotting	22	Gene regulation, signal transduction, development
Tanaka et al. (2005)	Differential display	10	Sugar metabolism, signal transduction, carotenoid biosynthesis, etc.
McGregor (2006)	cDNA microarray	975	Sucrose, starch and sporamin metabolism, protein synthesis and degradation, transport, signal transduction, cell division, transcription, etc.
Tao et al. (2012)	RNA sequencing	4,721	Starch and sucrose metabolism, cell division, regulation of transcription, membrane transport, stress response, etc.
Firon et al. (2013)	RNA sequencing	8,353	Carbohydrate metabolism, starch biosynthesis, lignin biosynthesis, transport, meristematic tissue identity and maintenance, etc.
Wang et al. (2015)	cDNA microarray	5,368	Starch synthesis, sugar signaling, abscisic acid signaling, protein amino acid dephosphorylation, lignin biosynthesis, protein amino acid phosphorylation, coumarin biosynthesis, fatty acid biosynthesis, auxin signaling, etc.

the gene expression using a cDNA microarray containing 3,072 sweetpotato cDNA sequences, and identified 975 genes showing differential expression between fibrous and storage roots. The data indicated upregulation of sucrose, starch, and sporamin metabolism in the storage roots, as well as upregulation of the genes involved in other physiological processes, such as protein synthesis and degradation, signal transduction, and cell division.

Recent progress made in sequencing technologies has enabled large-scale transcriptome analysis of non-model plants without genome sequence information. Tao et al. (2012) compared the transcriptomes of roots in different developmental stages, and found 4,721 transcripts that were differentially expressed between the fibrous roots and initial storage roots. They explained that the transcripts involved in starch and sucrose metabolism are enriched in the expanding storage roots as compared with the initial storage roots. They also found that the genes involved in cell division, regulation of transcription, membrane transport, and stress response are differentially expressed between roots in different developmental stages. Similarly, Firon et al. (2013) classified the young adventitious roots as either initiating storage roots or non-initiated fibrous roots by microscopic analysis, and identified 8,353 genes exhibiting differential expression between them. Their results indicated upregulation of the genes involved in carbohydrate and starch biosynthesis, and downregulation of the genes involved in lignin biosynthesis in the initiating storage roots, as well as strong upregulation of the sporamin and β-amylase genes. The data also indicated that the expressions of several regulatory genes involved in meristematic tissue identity and maintenance are elaborately controlled during the storage root initiation process. Wang et al. (2015) also analyzed the transcriptional profiles of sweetpotato roots in seven distinct developmental stages using a microarray containing 39,724 genes, and found 5,368 genes showing significant changes in gene expression during storage root development. The results suggested that starch biosynthesis is up-regulated and lignin biosynthesis is down-regulated during storage root development, along with changes in other biological processes, as summarized in Table 1.

Taken together, these gene expression studies revealed that thousands of genes are up- or down-regulated during the formation of storage roots in sweetpotato. Some changes in observed gene expression, such as upregulation of starch and storage protein biosynthesis and downregulation of lignin biosynthesis in the storage roots, closely match previous anatomical and physiological descriptions, suggesting that these gene expression studies are helpful for elucidating an outline of molecular events during the formation of storage roots. These gene expression studies also reported the changes in expression of several regulatory genes during the formation of storage roots. McGregor (2006) and Wang et al. (2015) reported the differential expression of auxin-responsible genes in fibrous and storage roots. Also, McGregor (2006) and Firon et al. (2013) reported the differential expression of ethylene-responsive factors. These results imply the involvement of auxin and ethylene in the formation of storage roots. Preferential expression of the homologues of the *Arabidopsis SHORT-ROOT* gene in fibrous roots was described by Tao et al. (2012), Firon et al. (2013), and Wang et al. (2015). As the SHORT-ROOT protein of *Arabidopsis* is involved in the radial patterning of roots (Helariutta et al. 2000), this gene is possibly involved in the determination of root architecture during the initial stage of adventitious root development. McGregor (2006) and Wang et al. (2015) reported differential expression of MADS-box family proteins, while Firon et al. (2013) and Wang et al. (2015) reported upregulation of class I knotted1-like homeobox (*KNOXI*) genes in the storage roots. The involvement of these genes in the formation of storage roots was also suggested in the molecular biological studies described below.

Functional analysis of specific genes involved in storage root formation

In addition to the large-scale gene expression analyses described above, functional analyses of specific genes differentially expressed during the formation of storage roots have been reported.

Tanaka et al. (2009) analyzed the physiological functions of *SRF1*, which encodes a Dof-type zinc finger transcription factor, by using transgenic sweetpotato plants. Storage roots of the plants overexpressing *SRF1* contained higher levels of starch in exchange for reduced glucose and fructose content. Further analysis of gene expression and enzyme activity in the storage roots of transgenic plants suggested that *SRF1* is involved in the regulation of carbohydrate metabolism during storage root formation through the modulation of vacuolar invertase gene expression.

Noh et al. (2013) reported functional characterization of an expansin gene (named *IbEXP1*) that is down-regulated in storage roots. The fibrous roots of transgenic sweetpotato plants with suppressed expression of *IbEXP1* were thicker than those of the control plants, and showed an enhanced proliferation of metaxylem and cambium cells and reduced lignification in the central stele. These transgenic plants showed enhanced storage root formation and drastically shortened fibrous roots between the storage roots and stem when cultured in soil, suggesting that *IbEXP1* plays a negative role in the formation of storage roots by suppressing the proliferation of cambium and metaxylem cells. *IbEXP1* is the first gene whose involvement in storage root morphogenesis has been shown directly by using soil-grown transgenic sweetpotato plants.

Several reports have also focused on the transcription factors with known functions in plant development. Kim et al. (2002, 2005) reported the isolation of MADS-box genes expressed in sweetpotato roots. Some of these MADS-box genes are expressed around the primary cambium of the developing storage roots. MADS-box genes encode transcriptional factors containing a DNA-binding motif called MADS-box. In addition to their well-established functions in flower development, several MADS-box genes are known to have roles in root development (see Yu et al. 2014 and references therein). Ku et al. (2008) isolated a MADS-box gene (named *IbMADS1*) expressed in young storage roots. *IbMADS1* is also expressed in the swollen roots induced by exogenous application of cytokinin and jasmonic acid. Transgenic potato (*Solanum tuberosum*) plants overexpressing *IbMADS1* showed a partial swelling of roots that was mainly attributed to a proliferation of metaxylem cells with thickened cell walls. Noh et al. (2010) reported functional characterization of a MADS-box gene (named *SRD1*) that was 99% identical to *IbMADS1* in the nucleotide sequence of the coding region. Expression of *SRD1* was observed in the cambium cells of the developing storage roots and regulated by exogenous auxins. The transgenic plants overexpressing *SRD1* cultured *in vitro* produced thicker and shorter fibrous roots, with increased metaxylem and cambium cells. These data strongly suggest that MADS-box genes are involved in the determination of root architecture at an early stage of storage root development.

Tanaka et al. (2008) and Tanaka (2010) found that four *KNOXI* genes (named *Ibkn1* to *Ibkn4*) are preferentially expressed in the storage roots as compared to the fibrous roots. As described above, expression of the *KNOXI* genes in the storage roots was also observed by Firon et al. (2013) and Wang et al. (2015). The *KNOXI* genes of plants encode transcription factors expressed in the shoot apical meristem (SAM) and are known to play important roles in plant development, especially in the maintenance of SAM activity (Hay & Tsiantis 2009). In *Arabidopsis thaliana*, *KNOXI* genes up-regulate cytokinin biosynthesis and down-regulate lignin biosynthesis (Mele et al. 2003, Yanai et al. 2005). A similar distribution of the *KNOXI* gene expression and cytokinin concentration was observed in sweetpotato storage roots (Tanaka et al. 2008), suggesting that the *KNOXI* genes may also be involved in regulation of cytokinin biosynthesis in the storage roots. Recent experiments showed that *Ibkn2* and *Ibkn4* tend to be highly expressed in the developing storage roots of cultivars with higher storage root formation capability (Tanaka 2010). A similar expression pattern was observed among F_1 progenies between sweetpotato and *I. trifida*. However, transgenic plants with suppressed *Ibkn2* expression produced storage roots similar to those of non-transgenic control plants (Tanaka et al. unpublished results). It is possible that *Ibkn2* and *Ibkn4* have redundant functions, and that the suppression of both genes is necessary to affect the storage root formation process. It has been clarified that *KNOXI* proteins, together with BEL1-like homeodomain proteins, play key roles in the induction of potato tubers (Rosin et al. 2003, Chen et al. 2003, 2004). It is possible that a similar molecular mechanism may also control the formation of storage roots in sweetpotato.

Together, these molecular biological and transgenic studies have begun to reveal the individual genes putatively involved in the characteristic physiological processes of storage root formation, such as the regulation of carbohydrate metabolism, lignification of the stele, determination of root architecture, and cambium cell development. However, the findings of these studies are still not sufficient to clarify the detailed physiological functions of these genes or the functional relationships among them. Further studies on their physiological functions and regulation mechanisms would contribute to understanding the molecular mechanisms underlying the formation of storage roots in sweetpotato.

Conclusions

As described above, molecular studies have identified numerous genes showing differential expression between developmental stages relative to the formation of storage roots. In addition, several genes have been suggested to have functions in the characteristic process of storage root formation based on molecular biological studies and analyses using transgenic sweetpotato plants. Although these studies are valuable as a first step in the molecular-level analysis of storage root formation, the information obtained remains fragmentary and has not revealed the full molecular mechanism regulating this complex process.

As the formation of storage roots appears to be a default process in sweetpotato root development, it is unclear whether a specific signal exists to initiate storage root development. Nonetheless, genetic differences should exist between sweetpotato and related plant species that do not produce storage roots. Recently, draft whole-genome sequences of two diploid *I. trifida* lines have been reported (Hirakawa et al. 2015), which would be useful resources for investigating the hexaploid genome (about 2.4 Gb) of sweetpotato. Because genome-wide DNA markers will be obtained using these genome sequences, molecular genetic analysis using *I. trifida* lines with different root thickening capabilities could lead to a major breakthrough in the genetic study of storage root formation. Whole-genome sequencing of Japanese morning glory (*I. nil*) is also ongoing. Comparative genome studies between these diploid *Ipomoea* species and sweetpotato may reveal the genetic differences related to storage root formation. However, to

elucidate the critical genes involved in storage root formation and understand the complex gene regulation network that controls this process, further genetic, physiological, and molecular biological studies are needed. The information and methodologies obtained in the model plants and other root crops will be helpful for these studies. In addition, the collaboration of researchers from various research areas, such as genetics, breeding, crop science, plant physiology, and molecular biology, is needed to better understand the genetic and molecular mechanisms underlying the formation of storage roots in sweetpotato, and thus further improve this vital crop.

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