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Use of Single-leaf Cutting in the Study of the Expression of Starch Synthesis and Modification Genes in Sweetpotato

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Abstract

The evaluation of source potential and sink strength is the generally large and laborious sample size required to adequately assess any one of the parameters in field-grown sweetpotato. For this purpose we used the rooted single-leaf cuttings with petioles, because the source and sink organs are restricted in this system. The rooted single -leaf cutting of sweetpotato provides a unique source-sink model system, and is established within about 50 days after planting. In this study, the sink potential of sweetpotato tubers was examined based on the expression of genes for starch synthesis (*AGPase*) and modification (*SBEII* and *GBSSI*) in single rooted leaf plant. The gene expression patterns of *GBSSI, SBEII* and *AGP*ase at various developmental stages and in different types of root tissues presented. These results suggest that the rooted single-rooted method can be used an ideal model system to study physiological and biochemical mechanisms in sweetpotato.

Key words: Sweetpotato (*Ipomoea batatas* (L.) Lam.), rooted single-leaf cuttings, source-Sink model

Introduction

The sweetpotato is an economically important crop. It is also an interesting plant because it can propagate from vegetative tissues, root tubers and vine cuttings, as well as from seed (Onwueme 1978). The tuberization process in sweetpotato is known to be controlled by environmental factors, such as photoperiod, temperature, oxygen concentration, moisture and soil properties (Onwueme 1978).

Anatomical characteristics have also been identified as the factors influencing the yield of sweetpotato. The high activity of the vascular cambium relative to that of anomalous secondary and testic shapertiary meristems resulted in production of the characteristic of high-yielding cultivars of tuber crops. The tuber width is the most important characteristic indicative of high yield in most tuber crops. The storage root has central pith (Lowe and Wilson 1974) and a vascular cambium that has a capacity to generate storage parenchymatous cells instead of xylem cells (Artschwager 1924; Wilson and Lowe 1973). Frustration of tuber initiation occurs in some roots by complete centripetal development of the primary xylem elements leading to lignification of the central stele (Wilson and Lowe 1973, Wilson 1982).

In sweetpotato, various components of sink activity have been identified as more important determinants of yield in addition to the photosynthetic capacity of the foliage (Fujise and Tsuno 1967; Hozyo 1977; Wilson 1982). However, the nature of the tuber sink potential is not clear and the genetic factors regulating the tuber sink potential are still unknown. Development of the sweetpotato storage root coincides with starch accumulation, and the genes that affect the synthesis and modification of starch and related metabolisms may play a central role in the control of storage root development and storage sink strength. Extensive studies on carbohydrate metabolism, at the molecular biology level, have been made using the potato as a model plant (Fernie and Willmitzer 2001; Fernie et al. 2002). However, a potato tuber is a modified stem, while the sweetpotato storage organ is a modified root. The documentation of information concerned with the expression of carbohydrate metabolism genes [*Granule Bound Starch Synthase* (*GBSS*), *Starch Branch Enzyme* (*SBE*) and *ADP-glucose pyrophosphorylase* (*AGPase*)] in sweetpotato may provide insight into the mechanisms specific for the starch metabolism in root crops.

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In sweetpotato, the tuber sink potential has been estimated by grafting a scion of another cultivar uniform in size on the stock cultivar (Nakatani et al. 1988) or the reciprocal grafting (Hahn 1997) in the field. However, the size of source and sink organs other than tuber is not restricted even in the graft with the scion uniform in size, because the top growth of grafts varies with the stock cultivar. In some plants, rooted single leaves have been used as source-sink model plants since the source potential is restricted (Humphries 1963; Sawada et al. 1986). In sweetpotato rooted leaves have also been used for the studies on the translocation of assimilate from leaves to roots (Kato et al. 1972), tuber cracking (Oyanagi et al. 1987), and for screening growth regulators (Spence and Humphries 1972; McDavid and Alamu 1980). Recently, Nakatani et al. (1988) reported a method of using single leaves grafted on rooted stocks for examination of source and sink potentials of different cultivars. However, the handling procedure is complicated and many problems arise during grafting of stock and scion, such as rot disease of petiole.

In the present study, we used rooted single-leaf cuttings with petioles, and examined the expression patterns of starch regulation genes (*GBSSI, SBEII* and *AGPase*) during development of storage root in the rooted single-leaf cuttings.

Materials and Methods

Plant materials

The virus-free sweetpotato (Kokei 14) was planted in plastic vats containing a mixture of vermiculite and sand (1:1, v/v). The vats were placed in a growth chamber. Expanded leaves were cut from the plants at the base of the petiole, and each petiole of the leaves was cut to uniform lengths of 60 mm to 70 mm. The single leaves with petioles were planted in soil and maintained under a 12-h light / 12-h dark light condition. The irradiance at the leaf surfaces was 200-300 μmol photons m^{-2} s^{-1} (400-700 nm), from fluorescent lamps. The temperature was 24℃ during the light and dark periods. The relative humidity was about 80%.

Ten rooted single leaves were sampled at 20, 30, 40, 50 and 60 days after planting, and divided into leaves with petioles and roots for analysis of fresh weight and gene expression.

RNA extraction and Reverse-Transcriptase (RT)-PCR

Transcript levels were measured either by RT-PCR. Total RNA was isolated from whole plants or root tissues RNA as described by Kim et al. (2002a), and treated extensively with *RNase*-free *DNase I* to remove any contaminating genomic DNA. The first-strand cDNA was synthesized using *Pfu* Turbo *polymerase* (Stratagene, La Jolla, CA) from 2 μg of total RNA in a 20-μL reaction volume, and 2 μL of the reaction mixture was subjected to subsequent PCR in a 50-μL reaction volume. *GBSSI* (*Granule Bound Starch Synthase I* Genbank accession number AB071976) (5' tggtcgtggttcttcttctgct3' and 5' tgtctccatgactgcgaagtt 3'), *SBEII* (*Starch Branch Enzyme I* Genbank accession number AB071286) (5' ccacggccaaaaagaccaaagtcac 3' and 5' accaccatcttgcgtttgagagcag 3'), *AGPase* (ADP-glucose pyrophosphorylase / Genbank accession number AJ252316) (5' ggggatgaagtggtttcaggg 3' and 5' gggactttt-ccatctgccagaagcg 3') and *Tublin* (5' caactaccagccaccaa-ctgt 3' and 5' caagatcctcacgagcttcac 3') were amplified using the indicated primers according to the following cycling conditions: RT-PCR for 25 cycles, depending on the linear range of PCR amplification for each gene, with each cycle at 94℃ or 0.5 min, 60℃ for 0.5 min, and 72℃ for 1 min, with a final cycle at 72℃ for 5 min to allow the completion of the polymerization.

Histological analysis

Transverse sections of petioles for light microscopy, 80 μm thick, were obtained using D.S.K. Microslicer DTK-3000 in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 at room temperature. Sections were mounted on glass slide with the same solution, and observed using a Nikon OPTIPHOTO-EDA microscope (Nikon, Japan).

Results and Discussion

We used the rooted single-leaf cuttings with petioles but without stems and nodal parts to investigate the mechanism of the initiation (emergence) and development of roots and expression pattern of the genes controlling starch synthesis and modification in sweetpotato.

In single-leaf cuttings with petiole, adventitious roots developed from the callus formed at the cut ends of the petioles (Figure 1A). The growth of the leaf and petiole was limited to restricted range because the leaf had already fully expanded at the time of planting (Figure 2). By contrast, the root, which is the only storage organ for accumulating assimilates from the leaf, grew continuously increasing the weight throughout the experimental period (Figure 2). The primary root emerged at 7 days after planting (day 7) (Figure 1A) and elongated to a length of 2-3 cm at day 8. Small lateral roots were observed at the upper part of the root on day 10. The sweetpotato roots were classified into

three types after day 20; fibrous white roots, thin pigmented roots and thick pigmented roots (Figure 1C). Thick- pigmented roots generally turn to tubers (Lowe and Wilson 1974). A thick root was defined as the root with uniform thickening, at least 2mm in diameter, along the root axis (Lowe and Wilson 1974). Such thick roots indicate the onset of secondary growth (Wilson 1982). The root length started to decease 40-50 days after planting, root weight increased significantly after day 40 and pigmented roots changed to storage roots (Figure 2). These facts indicated that initially the root thickened uniformly and then tuber bulking started on day 40-50.

Anatomical analysis of the sections of tubers developing from petiole of the leaf cuttings conformed to the pattern of cellular activity during primary root development. In the upper part of the petiole (Figure 3 A), there were four bundles; and the outer phloem in the two large median bicollateral bundles was composed of two or three strands. In the leaf cutting at day 10, secondary growth was observed in the petiole (Figure 3). The vascular cambium initiated in the parenchymatous zone through division of the single-layered pericycle, separating the xylem from the phloem, and was connected to form a continuous and irregular cylinder.

The roots developed from the petiole formed storage root as shown in Figure 1 F, suggesting that the roots functioned as the active sink during the experimental period, accumulated starch using the assimilate supplied from the leaf, and formed storage root (Figure 1 F). These results

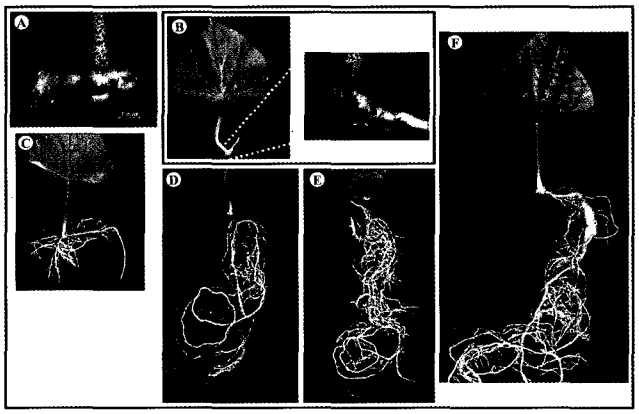

Figure 1. Rooting and growth of single-leaf cutting with petiole of sweetpotato (rooted single-leaf cutting). A, day 7; B, day 10; B, day 20; D, day 30; E, day 40; F, day 60 after planting

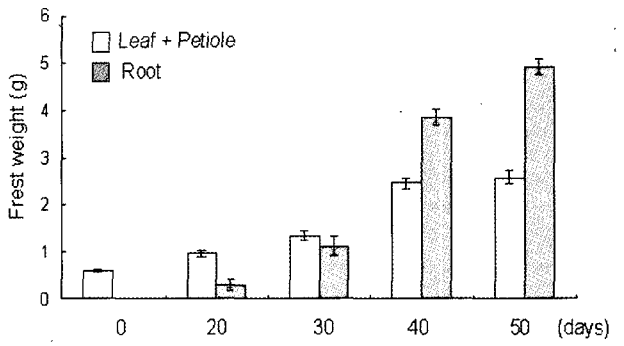

Figure 2. Changes in fresh weights of leaf+petiole and root in the single-leaf cuttings of sweetpotato during soil culture. Leaf+petiole and root fresh weights were measured in ten plants. Vertical lines show SE.

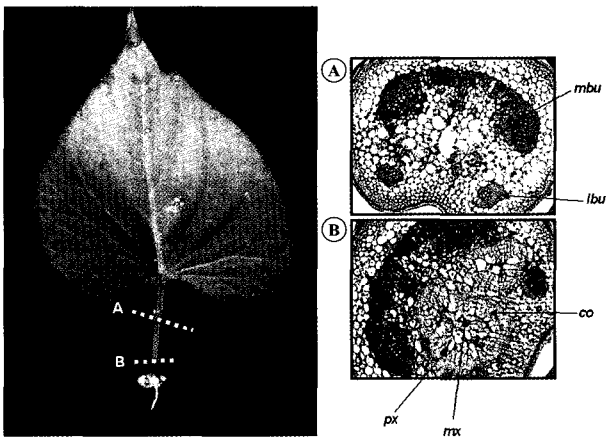

Figure 3. Transverse section of petiole at indicated site of rooted single-leaf cutting of sweetpotato at day 10 after planting. *co*, Cortex; *mx*, metaxylem; *px*, protoxylem; *lum*, Lateral bundle; *mbu*, median bundle.

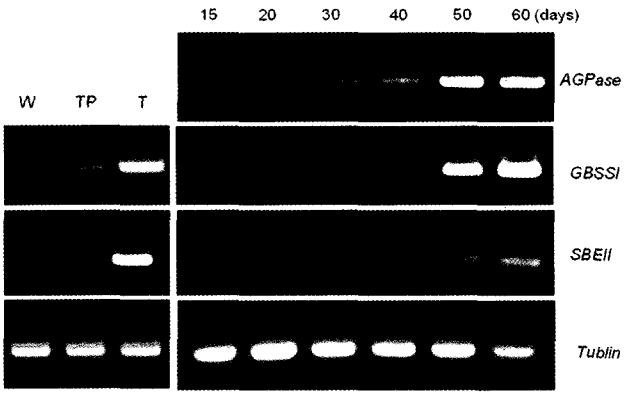

Figure 4. Expression pattern of *GBSSI*, *SBEII* and *AGPase*. A, Expression pattern of *GBSSI*, *SBEII* and *AGPase* of sweetpotato root [White fibrous roots (W), Thick pigmented roots (TP) and Storage roots (T)] sampled on day 60; B, Expression of genes in sweetpotato roots with passage of time. *Tublin* was used as a control.

suggest that the rooted single-leaf cutting of sweetpotato provides a unique source-sink mode system, and is established within about 50 days after planting.

Root bulking in sweetpotato is correlated with starch accumulation and surface pigmentation (Kim et al. 2002b). The initiation of tuber bulking in a small percentage of sweetpotato roots shows that the differentiation of the storage root from the root in sweetpotato is determined by the activities of starch synthesis and accumulation (Lowe and Wilson 1974). In this study, the fibrous white root, thick pigmented root and developing storage root of the same plants were compared for the expression of genes for starch synthesis and modification. RT-PCR rather than Northern hybridization was used to analyze the gene expression because RT-PCR is more sensitive than Northern hybridization for the detection of gene transcription (Li et al. 1988). RT-PCR analysis can effectively detect the transcripts of weakly expressed genes, whereas Northern hybridization can not (Tanaka et al. 1998). Figure 4 A shows the results of RT-PCR analysis. RT-PCR for *SBEII* and *GBSSI* produced RT-PCR products at expected size. Expression of the two genes (*GBSSI* and *SBEII*) for starch modification was strongest in developing storage root and was very low in fibrous white roots. The results indicated that the degree of expression of the two starch modification genes was the highest in storage root.

We compared the expression patterns of *GBSSI*, and *SBEII* with that of *AGPase* in sweetpotato roots. *AGPase* is a key enzyme for starch synthesis and is a marker of tuberization in sweetpotato, which means that *AGPase* is positively correlated with sink strength under normal conditions (Li and Zhang 2003). As shown in Figure 4 B, *AGPase* expression increased with time after planting. On the other hand, expression of *GBSSI* and *SBEII* was low during the initial 30-40 days after planting and increased at day 50. AGPase expression in sweetpotato root started at the initial stage of tuberization reaching a maximum at day 50. This result coincides with the report of Kim et al. (2002b) that *AGPase* mRNA was not detected in thickened pigmented roots, but was found in developing tuberous roots at 8 weeks after planting in field condition.

These results indicated that expression patterns of *GBSSI* and *SBEII* were related with the enlarging of the storage root, for which rapid accumulation of starch may be necessary. The gene expression patterns of *GBSSI, SBEII* and *AGPase* at various developmental stages and in different types of root tissues presented in this paper clearly indicate that activity of the genes related to starch synthesis and modification in storage roots is positively correlated with sink strength during sweetpotato root development.

The evaluation of source potential and sink strength is generally large and laborious sample size required to adequately assess any one of the parameters in field-grown sweetpotato. However, the rooted single-rooted method can be used to measure source potential and sink strength because it requires less material and time, suggesting that an ideal model system to study physiological and biochemical mechanisms of tuber formation.

References

Artschwager E (1924) On the anatomy of the sweetpotato root with notes on internal breakdown. J Agr Res 27: 157-166

Fernie AR and Willmitzer L (2001) Molecular and biochemical triggers of potato tuber development. Plant Physiol 127: 1459-1465

Fernie AR, Willmitzer L and Trethewey RN (2002) Sucrose to starch: a transition in molecular plant physiology. Trends Plant Sci 7: 35-41

Fujise K and Tsuno Y (1967) The effect of potassium on dry matter production of sweetpotato. In Proceeding of the International Symposium on Tropical Root Crops. Vol. 1. pp. 20-33. Univ. of the West Indies, Trinidad.

Hahn SK (1997) A quantitative approach to source potentials and sink capacities among reciprocal grafts of sweetpotato varieties. Crop Sci. 17: 559-562.

Hozyo Y (1977) The influences of source and sink on plant production of Ipomoea grafts. Jpn Agri Res Quarterly 11: 77-83.

Humphries EC (1963) Dependence of net assimilation rate on root growth of isolated leaves. Ann Bot 27: 175-183.

Kato S, Kobayasi H and Hozyo Y (1972) Translocation of ^{14}C-photosynthates in isolated sweetpotato leaves, *Ipomoea batatas* Poiret. Proc Crop Sci Soc Jpn 41: 147-154.

Kim SH, Mizuno K and Fujimura T (2002a) Isolation of MADS-box genes from sweetpotato (*Ipomoea batatas* (L.) Lam.) expressed specifically in vegetative tissues. Plant and Cell Physiol 43: 314-322.

Kim SH, Mizuno T, Sawada S and Fujimura T (2002b) Regulated expression of *ADP glucose pyrophosphorylase and chalcone synthase during* root development in sweetpotato. Plant Growth Regul 38: 173-179.

Li H, Glyllesten YB, Cui X, Saiki RK, Erlich HA and Arnheim N (1988) Amplification and analysis of DNA sequences in single human sperm and diploid cell. Nature 335: 414-417.

Li XQ and Zhang D (2003) Gene expression activity and pathway selection for sucrose metabolism in development storage root of sweetpotato. Plant Cell Physiol 44: 630-636.

Lowe SB and Wilson LA (1974) Comparative analysis of tuber development in six sweetpotato (*Ipomoea batatas* (L.) Lam) cultivars. 1. Tuber initiation, tuber growth and partition of assimilates. Ann Bot 38: 307-317.

McDavid CR and Alamu S (1980) The effect of growth regulators on tuber initiation and growth in rooted leaves of

two sweetpotato cultivars. Ann Bot 45: 363-364.

Nakatani M, Oyanagi A and Watanabe Y (1988) Tuber sink potential in sweetpotato (*Ipomoea batatas* (L). Lam.). Jpn J Crop Sci 57: 535-543.

Onwueme IC (1978) Sweeet potato. In The Tropical Tuber Crops. Edited by I.C. Onwueme, pp. 167-195. John Wiley and Sons, Chichester.

Oyanagi A, Nakatani M and Watanabe Y (1987) Studies on the factors inducing cracking in tuberous roots of sweetpotato (*Ipomoea batatas* (L). Lam.). Jpn J Crop Sci 56: 190-197.

Sawada S, Hayakawa T, Fukushi K and Kasai M (1986) Influence of carbohydrates on photosynthesis in single, rooted soybean leaves used as a source-sink model. Plant Cell Physiol 27: 591-600.

Spence JA and Humphries EC (1972) Effect of moisture supply, root temperature, and growth regulators on photosynthesis of isolated rooted leaves of sweetpotato (*Ipomoea batatas*). Ann Bot 36: 115-121.

Tanaka N, Yamakawa M and Yamashita I (1998) Characterization of transcription of genes involved in hairy root induction on pRil724 core T-DNA in two Ajuga reptans hairy root lines. Plant Sci 137: 95-105.

Wilson LA (1982) Tuberization in sweetpotato (*Ipomoea batatas* (L). Lam.). pp. 79-94 in Sweetpotato, edited by RL Villareal and TD Griggs. Asian Vegetable Research and Development Center, Shanhua, Twainan, Taiwan.

Wilson LA and Lowe SB (1973) The anatomy of the root system in west Indian sweetpotato (*Ipomoea batatas* (L). Lam.) cultivar. Ann Bot 37:633-643.