Cloning and Characterization of Sweetpotato *isoamylase* gene (*IbIsa1*) Isolated from Tuberous Root

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The cDNA clone of the sweetpotato *isoamylase* gene, encoding starch-debranching enzyme (*IbIsa1*) in sweetpotato (*Ipomoea batatas* (L.), cv. Kokei 14), was isolated and sequenced. The amino acid sequence of *IbIsa1* is 70% and 79% identical to that of *AtIsa1* from Arabidopsis and *StIsa1* from potato, respectively. DNA gel-blot analysis demonstrated that at least two copies *IbIsa1* are present in the sweetpotato genome. *IbIsa1* was found to be strongly expressed in tuberous root. The transcript level of *IbIsa1* in the root of rooted single-leaf cuttings was extremely low during the first 15 to 40 days after planting. The transcript levels continuously increased up to 50 days, at which time the tuberous root was almost completely developed. This indicates that *IbIsa1* may work in concert with *AGPase* large subunit, *GBSSI* and *SBEII* during the primary phase of starch granule formation.

Key Words: sweetpotato (*Ipomoea batatas* (L.) Lam.), *Isoamylase*, rooted single-leaf cuttings, starch.

Introduction

Sweetpotato is one of the most important crops in the world and provides not only staple food but also important industrial raw materials. Its starch is used as food and industrial resources. Starch, the most common form of stored carbon in plants, is composed of two types of α -1,4-linked glucan polymers: essentially unbranched amylose and regularly branched amylopectin. Amylopectin is synthesized via three committed enzyme steps: *ADP-Glc pyrophosphorylase*, which synthesizes sugar nucleotide precursors; starch synthase, which extends the α -1,4-linked glucan chains using ADP-Glc; and starch-branching enzyme, which introduces α -1,6 branch points to form amylopectin. However, the activity of starch-debranching enzymes, which hydrolyze α -1,6 branches in glucans, is also important for amylopectin synthesis (Hussain *et al.* 2003, Pan and Nelson 1984).

Early research on the maize *su1* mutant (Pan and Nelson 1984) implicated a role for debranching enzymes (DBE) in determining the chain length of amylopectin side-chains, one of the two components of starch. This conclusion is recently being corroborated by studies on the same mutant and mutants in other organisms. Biochemical analyses of *su1* mutants of maize (Rahman *et al.* 1988, James *et al.* 1995, Beatty *et al.* 1999), rice (Nakamura 1996, Nakamura *et al.* 1996, Nakamura *et al.* 1997) endosperm and *sta7* mutants of *Chlamydomonas* (Ball *et al.* 1996, Mouille *et al.* 1996) indicated that DBEs are involved in amylopectin biosynthesis. Before these studies, DBEs were considered to be mainly

catabolic enzymes active during the degradation of starch, such as seen in germinating seeds (Hussain *et al.* 2003).

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 (Wilson 1982, Fujise and Tsuno 1967, Hozyo 1977). 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 (Ferne Willmitzer 2001, Ferne *et al.* 2002). However, a potato tuber is a modified stem, while the sweetpotato storage organ is a modified root. The documentation of information on the expression of carbohydrate metabolism genes in sweetpotato may provide insight into the mechanisms specific for the starch metabolism in root crops.

We focused on the role of isoamylase-debranching enzymes in starch synthesis in developing sweetpotato tubers, and isolated a cDNA clone that encodes *isoamylase*, *IbIsa1*. *IbIsa1* is strongly expressed in developing tuberous root that synthesize storage starch, and its activity is positively correlated with sink strength during sweetpotato root development.

Material and Methods

Plant materials

Adult plants of sweetpotato (*Ipomoea batatas* (L.), cv.

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Kokei 14) grown in the field were harvested to analyze *IbIsa1* in different organs and for Southern hybridization.

Isolation of Isoamylase gene from sweetpotato

We extracted RNA using a modified version of the CTAB method (Kim *et al.* 2002a).

Conserved sequences in MADS box genes were generated using GENETYX (Software Development Co., Japan). One degenerated primer was designed according to the conserved amino acid sequence SINFVCAHDGFTLADLVITYN (GTITGYGCICAYGAYCGITTYACIYTIGC) in an alignment of the *isoamylase* sequence (Fig. 1A). First strand cDNA was synthesized from tuberous roots according to the manufacturer's instructions accompanying the SMART RACE cDNA Amplification Kit (Clontech, USA) and amplified with templates of cDNA using a degenerated primer and adaptor primer.

The remaining 5'-sequence of cDNA was obtained by 5'-RACE with total RNA of developing tuberous roots.

Southern hybridization

The CTAB method (Kim *et al.* 2002a) revised for extraction of genomic DNA from sweetpotato was used. Genomic DNA (about 10 µg) was restricted with *EcoRI*, *BamHI* and *HindIII*, separated on a 0.8% agarose gel, and transferred onto Hybond-N⁺ nylon membranes (Amersham Pharmacia, USA). Filters were hybridized with *isoamylase* specific probe labeled using DIG direct labeling reagent (Amersham Pharmacia, USA). Hybridization, washing and detection were performed according to the instruction manual of DIG labelling and detection system with CDP star (Amersham Pharmacia, USA).

RNA extraction and Reverse-Transcriptase (RT)-PCR

Transcript levels were measured by RT-PCR. Total RNA was isolated from whole plants or root tissues 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, USA) 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. *IbIsa1* (5' gtggccaggaggagagagttt 3' and 5' ccac cgataaccacctcggtc 3'), *AGPase* large subunit (*ADP-glucose pyrophosphorylase* large subunit/Genbank accession number AJ252316) (5' cggggatgaagtgttcagg 3' and 5' gggacttt tccatctgccagaagcg 3') and *Tublin* (5' caactaccagccaccaactgt 3' and 5' caagatctctcagagcttcac 3') were amplified using the indicated primers according to the following cycling conditions: RT-PCR for 25 or 30 cycles, depending on the linear range of PCR amplification for each gene, with each cycle at 94°C for 0.5 min, 60°C for 0.5 min, and 72°C for 1 min, with a final cycle at 72°C for 5 min to allow the completion of polymerization.

DNA sequencing and analysis

Both strands of the plasmid of positive clones were sequenced using a dye-terminator cycle sequencing kit, and an automated capillary DNA sequencer (Genetic Analyzer ABI 3100, PE Applied Biosystems, U.S.A.). All sequencing data were analyzed using the CLUSTAL-W program (<http://www.ebi.ac.uk/clustalw/>). Homology of amino acid sequences was analyzed using CLUSTAL-W program against sequences in the GeneBank and EMBL DNA database. The phylogenetic tree was constructed using the CLUSTAL-W program (<http://www.ebi.ac.uk/clustalw/>).

Results

Isolation of sweetpotato Isoamylase (IbIsa1)

Total RNA was isolated from developing tuberous roots of the plants 50 days after planting. This RNA was subjected to RT-PCR using degenerated *isoamylase* primers, starting with asymmetric amplification on the first strand cDNA (synthesized from the poly A tail) with the degenerated primer of conserved region. Bands between 600–800 bp were subcloned, and found to contain almost exclusively an *isoamylase* fragment. The remaining 5'-sequences of the three cDNAs were obtained by 5'-RACE with total RNA of developing tuberous roots. The sweetpotato *isoamylase* (Genebank accession number DQ074643/*IbIsa1*) full length cDNA was 2510 bp long. The 5- and 3-non-coding regions were 67 and 147 bp, respectively. This cDNA was most similar to the potato (*StIsa1*) and Arabidopsis *isoamylase* (*AtIsa1*) (Fig. 1A). Southern blot analysis under stringent conditions against sweetpotato genomic DNA revealed over two bands when digested with *EcoRI*, *HindIII* and *BamHI* (Fig. 3). The number of hybridizing bands indicates that the presence of at least two copies of *IbIsa1* in the sweetpotato genome, suggesting that there are more copies of *isoamylase* in the sweetpotato genome which is a hexaploid plant. It is unclear at present whether this result is due to the hexaploid and heterozygous nature of sweetpotato.

Sequence Analysis of IbIsa1

The deduced amino acid sequences of the *IbIsa1* cDNA suggest that they encode polypeptides of 785 amino acid residues (Fig. 1A). This is within the range of sizes reported for other tuber crop sequences: 793 residues in potato *isoamylase* (Hussain *et al.* 2003), 783 in Arabidopsis *isoamylase* (Gene accession number At2g39930). The primary structures of *IbIsa1* were aligned with those of other *isoamylases* in the *isoamylase* family (Fig. 1A). All *isoamylase* members share a high degree of amino acid identity (92%–94%) in the central portion of their amino acid sequences. The amino acid sequence of *IbIsa1* is 70% and 79% identical to that of *AtIsa1* from Arabidopsis and *StIsa1* from potato, respectively. Apart from a few stretches of sequence identity and similarity, the N termini of the *isoamylase* sequences are divergent (Fig. 1A).

An alignment of the predicted amino acid sequences

A

IbIsa1	MELLHSPSISQYSPKLSRSSNIRKTLGLSFGFPHNYFAEFKKARPSALAVNAIDGGG-	59
StIsa1	MELLHSPSISQYSPKLSRSSNIRKTLGLSFGFPHNYFAEFKKARPSALAVNAIDGGG-	54
AtIsa1	MDAICSSSLHHTKLNTLFS--NHTFFKISAPN--FKPLFRPISIS-----AKDRS-	49
IbIsa1	---ESDTAVVVEKPLPYGLLRFEVLSGHPAPFGATARDGGINFAVSSNATSAALCLLI	115
StIsa1	GVVKAATAAVVVEKPT--TERCRFEVLSSKPLPFGATATDGGVNFVAFSRNATAATLCLLI	112
AtIsa1	---NEAENIAVVEKPLK---SDRFISDGLSPFGPTVRDGVNFVSVYSTNSVATLCLLI	103
IbIsa1	SLADLPEKKVTEQIPLDPSINKTGDVWHVFLQDGFNDMLYGYSGDFGKFAPEEGHYFDSAR	175
StIsa1	TLSDLPKRVTEQIPLDPLANKTGDVWHVFLQDGFNDMLYGYSGDFGKFAPEEGHYFDSQ	172
AtIsa1	SLSDLRQNKVTEQIPLDPSRNRTGWHVFLRGDFKMDLYGYRFDGKFSPEEGHYDSSN	163
IbIsa1	ILLDPYAKAIVSRAEFGALGPEKDCWPFMACMLPTA-DKFDWEGDLPLKFFQRLDIYEM	234
StIsa1	IVLDPYAKAIVSRGEYGVLFEDDOWPFMAGMVFASDQDFWEGDLPLKFFQRLDIYEM	232
AtIsa1	ILLDPYAKAIVSRDEFGLVDFDNDWPFMAGMVFTRREEFDWEGDMHLKLPQKLDIYEM	223
IbIsa1	HVRGFTNHSSSETTEFGTYGVVEKLDHLKLVGNVCIELMPCHEFNELEYYSYNVFLGDY	294
StIsa1	HVRGFTNHSSSETTEFGTYGVVEKLDHLKLVGNVCIELMPCHEFNELEYYSYNVFLGDY	292
AtIsa1	HVRGFTNHSSSETTEFGTYGVVEKLDHLKLVGNVCIELMPCHEFNELEYYSYNVFLGDY	283
IbIsa1	KVNFVGYSYVNFSPMGYSAGMHKSLGAIDEFKYLVREAHRRGIEVIMDVFNHTAE	354
StIsa1	KVNFVGYSYVNFSPMGYSAGMHKSLGAIDEFKYLVREAHRRGIEVIMDVFNHTAE	352
AtIsa1	KVNFVGYSYVNFSPMGYSAGMHKSLGAIDEFKYLVREAHRRGIEVIMDVFNHTAE	343
IbIsa1	GNENGPMFSFRGVDSVFFMLAPKGEFFNYSGOGNTFNCNHVVARQFIFELRAYVWIEHM	414
StIsa1	GNENGPMFSFRGVDSVFFMLAPKGEFFNYSGOGNTFNCNHVVARQFIFELRAYVWIEHM	412
AtIsa1	GNENGPMFSFRGVDSVFFMLAPKGEFFNYSGOGNTFNCNHVVARQFIFELRAYVWIEHM	403
IbIsa1	VDGFFRDLASILTR-----VAGDMLTTGAPLSFPLIDMISSDPILRGVK-	460
StIsa1	VDGFFRDLASILTRSSSSWNAVNYGNSIDGVITTTGTPILTPPLIDMISSDPILRGVK-	471
AtIsa1	VDGFFRDLGSISSSLWDAANVYGAOVEDLLTGTPTISCPPIVIMISDPILRGVK-	462
IbIsa1	LIAEAWDCGGLYQVGAFFHWGIWSEWNGKYRDIVRQFIKGTDFGSGAFACLOGSPNLQY	520
StIsa1	LIAEAWDCGGLYQVGMFFHWGIWSEWNGKYRDMVRQFIKGTDFGSGAFACLOGSPNLQY	531
AtIsa1	LIAEAWDAGGLYQVGMFFHWGIWSEWNGKFRDVRQFIKGTDFGSGAFACLOGSPNLQY	522
IbIsa1	EGGRKPNWISINFCVCHDGTFLADLVTVYNNKHNMANGEDNKGDNHNNWNCGEQGFASI	580
StIsa1	EGGRKPNWISINFCVCHDGTFLADLVTVYNNKHNLANGEENNDGNHNNWNCGEQGFASI	591
AtIsa1	EGGRKPNWISINFCVCHDGTFLADLVTVYNNKHNLANGEENNDGNHNNWNCGEQGFASI	581
IbIsa1	SVKRLRRQMRNFFFLCLMVSGQVPMIYMGDEYGHGTGGNNNTYCHDNYNIFVWDKKDES	640
StIsa1	SVKRLRRQMRNFFFLCLMVSGQVPMIYMGDEYGHGTGGNNNTYCHDNYNIFVWDKKDES	651
AtIsa1	SVKRLRRQMRNFFFLCLMVSGQVPMIYMGDEYGHGTGGNNNTYCHDNYNIFVWDKKDES	641
IbIsa1	STOFFRFFCHVTKFRHEAESLGLDFFTAERLQWGHGTPGMDFWESSRFVAFITLVKVK	700
StIsa1	SSDFLRFCGLMTFRHECESLGLDGFPTAERLQWGHGTPGMDFWESSRFVAFITLVKVK	711
AtIsa1	HSDFRFFCHVTKFRHECESLGLDFFTAERLQWGHGTPGMDFWESSRFVAFITLVKVK	701
IbIsa1	GEIYIAFNASHLPVTITLPERGGYRNEPLVDTGKQTPFDLGDVPEKKTALKQYAHFLD	760
StIsa1	GEIYIAFNASHLPVTITLPERGGYRNEPLVDTGKQTPFDLGDVPEKKTALKQYAHFLD	771
AtIsa1	KEIYIAFNASHLPVTITLPERGGYRNEPLVDTGKQTPFDLGDVPEKKTALKQYAHFLD	761
IbIsa1	ANMYPMSYSYSSIIILLCPDEMIENS	785
StIsa1	ANMYPMSYSYSSIIILLSSADDA---	793
AtIsa1	ANMYPMSYSYSSIIILLSPKIDP---	783

C

	IbIsa1	AtIsa1	AtIsa2	AtIsa3	HvIsa1	HvIsa3	StIsa1	StIsa2	StIsa3	Su1
IbIsa1	100									
AtIsa1	70	100								
AtIsa2	26	26	100							
AtIsa3	44	42	31	100						
HvIsa1	65	65	26	41	100					
HvIsa3	43	42	30	67	43	100				
StIsa1	79	72	28	44	66	43	100			
StIsa2	29	28	52	31	29	28	100			
StIsa3	42	42	29	67	41	66	44	29	100	
Su1	66	65	26	42	81	43	68	27	42	100

of *IbIsa1* obtained using CLUSTAL W was used to search the Homologous Structure Alignment Database (Mizuguchi *et al.* 1997) using FUGUE (Shi *et al.* 2001). The top hit was the catalytic domain of *isoamylase* from *Pseudomonas amyloclavata* (residues 163 to 591) (Katsuya *et al.* 1998), with clear structural homology (Z score = 57.32, with Z score ≥ 6.0 signifying $\geq 99\%$ confidence). This result is consistent with the conformation of the *IbIsa1* to the structural requirements of members of the α -amylase superfamily of $(\alpha\beta)_8$ barrel proteins (Fig. 2). *IbIsa1* contained eight regions of β -strand, each followed by the regions of α -helix. Between β -strand 3 and 4 and between β -strands 6 and 7, there were additional regions of α -helix in each protein, as has been reported in other starch hydrolases of the α -amylase superfamily (Matsuura *et al.* 1984, Buisson *et al.* 1987, Jespersen *et al.* 1993, Klein *et al.* 1992).

The predicted peptide sequence for *IbIsa1* was used to screen the sequence databases for homologous sequences from other plants. *IbIsa1* is most similar to *AtIsa1* from Arabidopsis and the *StIsa1* gene product from potato. These align most closely with *IbIsa1*, *StIsa1* and *AtIsa1*, showing that all of these proteins belong to the *Isa1* subgroup of *isoamylases* in plants which fits well with the similarities in gene expression patterns of *IbIsa1* and *StIsa1* (Fig. 1B). These alignments of the isoform types are present in both monocots and dicots and they fall into structurally distinct isoform classes (Fig. 1B).

Expression patterns of *IbIsa1*

Total RNA was isolated from leaf, petiole, stem, and root tissues, and analyzed with the *IbIsa1* specific primer. To determine the local expression pattern of *IbIsa1*, we examined the expression of the gene in RNA from each organ. RT-PCR revealed that each gene is expressed in petiole, stem, leaf and tuberos root, but only weakly in in white fibrous root and thick pigmented root (Fig. 4A). Hussain *et al.* (2003) reported that the *isoamylase* in potato was expressed in both tubers and leaves. This result agrees with our finding

Fig. 1. Phylogenetic relationship among *isoamylase* isoforms. A. Alignment of fragments of peptide sequences of *isoamylases*. De-generated primer site is shown by dashes and box. B. Distance between deduced amino acid sequences of plant *isoamylase* isoforms determined by the CLUSTAL W program (<http://www.ebi.ac.uk/clustalw/>). C. Dendrogram representation of the prediction in B. The dendrogram was generated by the programs CLUSTAL-W program (<http://www.ebi.ac.uk/clustalw/>). *StIsa1*, *StIsa2* and *StIsa3* from potato are AY132996, AY132997 and AY132998, respectively. *AtIsa1* is At2g39930, *AtIsa2* is At1g03310, and *AtIsa3* is At4g09020 from Arabidopsis. *Taisa1* is AF438328 from wheat. *HvIsa1* (AF142589) and *HvIsa3* (BAD89532) are an *isoamylase* identified from barley, *Su1* is the product of the *sugary1* gene (U18908), and *ZmIsa2* (AAO17048) and *ZmIsa3* (AAO17049) from maize, and *CrIsa1* is deduced from EST AV630278 from *Chlamydomonas*.

Fig. 2. Alignment of the predicted protein sequences of *IbIsa1* with the *isoamylase* from *P. amylocleramosa*. Protein sequences were aligned using CLUSTAL W and FUGUE (<http://www-cryst.bioc.cam.ac.uk/~fugue/>). Regions of β -strand in *P. amylocleramosa* isoamylase (*Paisa*) are marked (b) below the alignment, regions of α -helix (a) below the alignment, and regions of 3/10 helix by (3) below the alignment.

that *IbIsa1* was expressed in both storage starch- and transitory starch-synthesizing tissues.

To elucidate more precisely the relationship between tuber development and the increased expression and temporal expression, we determined *IbIsa1* expression pattern in root tissues from single rooted leaf method (Kim *et al.*

Fig. 3. Southern-blot analysis of genomic DNA from sweetpotato. Genomic DNA (10 μ g per lane) of Elegant summer (E), Koganesengan (K), Kokei 14 (KO) and White star (W) was digested with *EcoRI*, *HindIII* and *BamHI*, fractionated on a 1% (w/v) agarose gel, and transferred to a nylon membrane. The membrane was hybridized with DIG-labelled *IbIsa1*-specific probe containing *EcoRI* digested fragments spanning about 230 amino acid of the C-terminus plus the 3' untranslated region. The size of marker fragments of DNA is indicated in kb.

Fig. 4. RT-PCR analysis of the *IbIsa1* gene activity in sweetpotato plants. Total RNA was isolated and used for RT-PCR analysis with gene specific primers as indicated for *IbIsa1* and *AGPase* large subunit. A. Each organ of sweetpotato at 5 months after planting. L, leaf; P, petiole; S, stem; W, white fibrous root; TP, thick pigmented root; T, tuberous root. B. Sweetpotato roots harvested at the indicated days after planting. *Tubulin* was used as a control.

2002b). Sweetpotato *AGPase* large subunit (*ADP-Glc pyrophosphorylase*) (Bae and Liu 1997, Kim *et al.* 2002c), was used as references. Interestingly, the *IbIsa1* transcript levels were exceedingly low during the first 20 days to 40 days. The transcript levels continuously increased up to 50 days, at which time the tuberous root was almost completely developed. The temporal expression pattern of the *IbIsa1* was exactly coincided to that of the *AGPase* large subunit. The expression pattern is similar to that of *SBEI* (*Starch Branching Enzyme I*) (unpublished data). Thus, the tissue specificity and temporal expression of *IbIsa1* during root development are similar to that previously reported for the *GBSSI* (*Granule-bound Starch Synthase I*), *SBEII* (*Starch Branching Enzyme II*) (Kim *et al.* 2005) and in concert during the stages of starch granule formation,

Discussion

cDNA clones encoding *isoamylase*-type starch-debranching enzyme (*IbIsa1*) were isolated from sweetpotato. The predicted products of *IbIsa1* are similar to other plant *isoamylases*, and the proteins belong to the α -*amylase* superfamily. Genes that encode *isoamylase* also can be identified Arabidopsis and potato (Goff *et al.* 2002, Yu *et al.* 2002, Hussain *et al.* 2003). By phylogenetic analysis, we found that *IbIsa1* is highly homologous to that *StIsa1* from potato and *AtIsa1* from Arabidopsis. We classified *IbIsa1* protein as a new gene of *Isa1* subgroup. After searching the Genebank from different higher plants, we found that *IbIsa1* is produced in most, if not all, monocots and dicots and that they represent the major types of *isoamylase* produced in angiosperms.

Structural analysis revealed that *IbIsa1* was highly similar to the *Su1* protein of maize that is associated with sugary phenotypes when mutated and to other *isoamylases* from potato. Mutants of *sugary1* of maize, *sugary* of rice, and *notch2* of barley have reduced storage starch synthesis in endosperm and accumulate a highly branched, water-soluble polysaccharide, phytoglycogen (Pan and Nelson 1984, Burton *et al.* 2002). We compared the primary amino acid sequences of the *IbIsa1* with those of *isoamylase* from *P. amylocladus* (Fig. 2). Differences in loop lengths between β -strands and α -helices provide specificity for substrate binding in different members of the $(\alpha\beta)_8$ barrel *starch hydrolase* superfamily (MacGregor 1993).

The results of RT-PCR revealed high activity of the *IbIsa1* gene in tuberous roots. However, the *IbIsa1* gene was poorly expressed in the white fibrous root and thick pigmented root (Fig. 4A). These findings indicate that the main role of *isoamylase* is the starch formation in the sink tissues during sweetpotato root development. However, expression of the *IbIsa1* gene was also detected in leaf, petiole, and stem tissues (Fig. 4A). Whether *isoamylase* in sink organs has a function or activity different from that in source tissues in sweetpotato remains unknown. Are there other isoforms of *isoamylase* in sweetpotato? Two types of *isoamylases*

(with a molecular mass of 68 and 140 kDa) were purified from developing maize kernels (Doehlert and Knutson 1991) and one type (with a monomeric molecular mass of 80–95 kDa) from potato tubers (Ishizaki *et al.* 1983). Southern hybridization suggested the presence of at least two copies of *IbIsa1* in Kokei 14. The band patterns in *HindIII* digestion within different cultivars (Koganesengan, White star and Elegant summer) (Fig. 3) are different. It is unclear at present whether this result is due to the hexaploid and heterozygous nature of sweetpotato. However, it would be interesting to clarify the homology between these copies of *IbIsa1* and whether they have the same strength and expression pattern.

AGPase large subunit is a key enzyme for starch synthesis and is a marker of tuberization in sweetpotato, which means that the *AGPase* large subunit is positively correlated with sink strength under normal conditions (Kim *et al.* 2002c). Expression of the *AGPase* large subunit expression in sweetpotato root started at the initial stage of tuberization reaching a maximum at day 50, consistent with the report of Kim *et al.* (2002c). As shown in Fig. 4 B, expression of the *AGPase* large subunit increased with time after planting. On the other hand, expression of *SBEI* (*Starch Branching Enzyme I*), *SBEII* (*Starch Branching Enzyme II*) and *GBSSI* (*Granule-bound Starch Synthase I*) was low during the initial 30–40 days after planting and increased at day 50 (Kim *et al.* 2005). These results suggest that *IbIsa1* works in concert with *AGPase* large subunit, *GBSSI*, *SBEI* and *SBEII* during the primary phase of starch granule formation.

In the present work, we isolated the *IbIsa1* gene and demonstrated that the synthesis and expression pattern of the gene during root development were similar to those of *isoamylase*. Further studies on the regulation of *IbIsa1* expression in developing tuberous root in transgenic plants are needed.

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