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Expression of Jerusalem artichoke (*Helianthus tuberosus* L.) fructosyltransferases, and high fructan accumulation in potato tubers

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Abstract

Fructans are polymers of fructose that are present as storage carbohydrates in various plants. Jerusalem artichoke (*Helianthus tuberosus* L.) contains a high amount of inulin. Two enzymes are involved in inulin biosynthesis. The sucrose:sucrose 1-fructosyltransferase (1-SST) enzyme mainly catalyzes the synthesis of 1-kestose from sucrose. In the next step, fructan:fructan 1-fructosyltransferase (1-FFT) catalyzes the synthesis of inulin from 1-kestose. In this study, the *Ht1-SST* and *Ht1-FFT* genes were isolated from Jerusalem artichoke and expressed in potato (*Solanum tuberosum* L.), either separately or together, via *Agrobacterium*-mediated transformation. Transgenic potato tubers overexpressing *Ht1-SST* accumulated 1-kestose to a high level (up to 3.36 mg/g), while tubers overexpressing both *Ht1-SST* and *Ht1-FFT* accumulated up to 3.14 mg/g short-chain inulin-type fructans, with the degree of polymerization (DP) ranging from 3 to 5, excluding high DP inulins. Transgenic potato plants accumulated fructo-oligosaccharides to a high level, following the fructan biosynthetic pathway of Jerusalem artichoke, and therefore present a high potential for the mass production of inulin through established potato breeding and cultivation methods.

Keywords: Inulin, *Solanum tuberosum*, Fructan, 1-Kestose, Sucrose:sucrose 1-fructosyltransferase, Fructan:fructan 1-fructosyltransferase

Introduction

Fructans are sucrose-derived water-soluble fructose polymers that commonly function as storage carbohydrates in more than 40,000 higher plant species including approximately 15% of flowering species, many belonging to the Poaceae, Liliaceae, and Asteraceae families [1]. Fructans are categorized into several groups, depending on their length (degree of polymerization [DP]), linkage type, and branching between fructose and glucose units [2]. The linear levan-type fructans consist of β-(2,6) linkages and are synthesized from 6-kestotriose in grasses such as *Phleum pratense* and *Poa secunda* [3, 4]. Graminan-type fructans consist of β-(1,2) and β-(2,6) linkages and are found in cereal crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) [5]. The most studied group of fructans is the linear inulin-type fructans. Inulins consist of linear β-(1,2) linkages with a terminal glucose residue and are present in Asteraceae family members such as chicory (*Cichorium intybus* L.), sunflower (*Helianthus annuus* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.) [6]. Inulins are synthesized from sucrose by the action of two different fructosyltransferases, sucrose:sucrose 1-fructosyltransferase (1-SST; EC 2.4.1.99) and fructan:fructan 1-fructosyltransferase (1-FFT; EC 2.4.1.100) [7]. The 1-SST enzyme catalyzes the biosynthesis of the trisaccharide 1-kestotriose (DP3) from a sucrose molecule, while 1-FFT catalyzes the elongation of 1-kestotriose to inulins containing additional β-(1,2)-linked fructose units with higher DP [6, 8]. On the other hand, 6G-fructosyltransferase (6G-FFT; EC

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2.4.1.243) is a key enzyme that catalyzes the synthesis of the inulin neoseries using 1-kestose as a fructose donor [9]. Recently, 6G-FFT was isolated from ryegrass (*Lolium perenne* L.) and functionally characterized [2]. Lp6G-FFT and native onion (*Allium cepa* L.) 6G-FFT exhibit 1-FFT activity and other similar properties [2, 10].

Jerusalem artichoke and chicory are the major industrial sources of inulin [11]. Jerusalem artichoke tubers contain a large amount of inulin and are therefore a valuable biomaterial for inulin production; 85% of the tuber dry weight corresponds to inulin-type fructans, with an intermediate DP (3–35 fructosyl residues) [6, 12]. Transgenic petunia (*Petunia hybrida* Vilm) plants overexpressing the *Ht1-SST* gene show the accumulation of low DP fructans, and the F1 progeny of a cross between two transgenic lines overexpressing *Ht1-SST* and *Ht1-FFT* show increased fructan content [13]. The chain length distribution of inulin-type fructans for globe artichoke (*Cynara scolymus*) and Jerusalem artichoke show no differences in the transient tobacco protoplast system [14, 15] as well as in transgenic maize (*Zea mays* L.), sugar beet (*Beta vulgaris* L.), and potato plants (*Solanum tuberosum* L.) [16, 17].

Inulin is a soluble dietary fiber with many health benefits. The addition of inulin in the diet helps to decrease lipogenesis in the liver [18], and oligofructose-enriched inulin prebiotics affect calcium absorption and bone mineralization [19]. Inulin-type prebiotics have also been reported to affect diarrhea and cell propagation in piglets [20]. Furthermore, inulin is used as a biomaterial for syrup and bioethanol production [21]. The successful industrialization of inulin products depends on cost-effective mass production technologies. Although inulin is abundant in Jerusalem artichoke tubers, its production is limited by the high cost. Transgenic crops may have a substantial advantage for inulin production worldwide using established breeding and cultivation protocols [17].

Potato is widely used for food and industrial purposes. We envision that potato plants engineered to produce inulin-type fructans will contribute to a broad spectrum of commodities in both the agricultural and industrial sectors. Here, we successfully induce overexpression of the *Ht1-SST* and *Ht1-FFT* genes in potato and confirm the production of inulin-type fructans in transgenic potato plants.

Materials and methods

Isolation of *Ht1-SST* and *Ht1-FFT* genes

Total RNA was isolated from the tubers of Jerusalem artichoke (*Helianthus tuberosus* cv. PJA) using the acid guanidinium thiocyanate-phenol–chloroform extraction method [22, 23]. First-strand cDNA was synthesized from 1 μg total RNA using AccuPower CycleScript RT PreMix (dT20) (Bioneer Co., Daejeon, Korea). Full-length *Ht1-SST* cDNA (1893 bp; GenBank accession number: AJ009757) was amplified from the cDNA template by PCR using primers HtSST_F (5′-AAAACCCTCCCT CAGGCCAC-3′) and HtSST_R (5′-CCATCAAAGTTC GAAAGTCC-3′) with the Expand Long PCR Kit (Roche Applied Science, Mannheim, Germany). Full-length *Ht1-FFT* cDNA (1848 bp; GenBank accession number: AJ009756.1) was amplified by PCR using primers HtFFT_F (5′-GTCAGTCACCATGCAAACCC-3′) and HtFFT_R (5′-AAAGGATAGCGATAGCCGG-3′). After sequence verification, the *Ht1-SST* and *Ht1-FFT* PCR products were cloned into the pCR8/GW/TOPO vector containing *attL* recombination sites using the pCR8/GW/TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA), generating the Gateway entry clones of *Ht1-SST* and *Ht1-FFT* (Fig. 1a). The pEarleyGate103 (pEG103) vector containing the *bar* gene (as a selectable marker), the *green fluorescent protein* (*GFP*) reporter gene, and a 6x-His-tag was used as the Gateway destination vector containing *attR* recombination sites to generate pEG103/1-SST and pEG103/1-FFT expression constructs. These constructs were introduced into *Agrobacterium tumefaciens* strain GV3101, as described previously [24], which were then used for potato transformation.

Plant material and transformation

Potato (*Solanum tuberosum* L. cv. Desiree) plants were cultivated in an incubation room at 24 °C, 16 h light/8 h dark photoperiod, and 50% relative humidity using aseptic technique. Stable potato transformation was performed by the cocultivation of leaf disc segments with *Agrobacterium* carrying the pEG103/1-SST and pEG103/1-FFT expression constructs, as described previously [25] (Fig. 1b). Transgenic shoots were induced on Murashige and Skoog (MS) medium containing 2.0 mg/L zeatin, 0.01 mg/L naphthalene acetic acid (NAA), 0.1 mg/L gibberellin (GA_3), 500 mg/L carbenicillin, and 1.0 mg/L Basta. Roots were induced on MS medium containing 1.0 mg/L Basta and 500 mg/L carbenicillin for the selection of transgenic plants. Positive transformants displaying Ht1-SST activity were selected, and the *Ht1-FFT* gene was introduced in *Ht1-SST* transgenic potato plants to generate transgenic plants co-expressing *Ht1-SST* and *Ht1-FFT* (hereafter referred to as SnF plants). SnF transgenic potato plants expressing *Ht1-SST* and/or *Ht1-FFT* genes were selected based on antibiotic resistance, and then selected primarily by genomic DNA PCR using Plant Direct PCR system (NanoHelix Co., Daejeon, Korea) with full length SST and/or full length FFT primers. Expression of SST and/or FFT genes of transgenic plants at the RNA level was confirmed using RT-PCR and

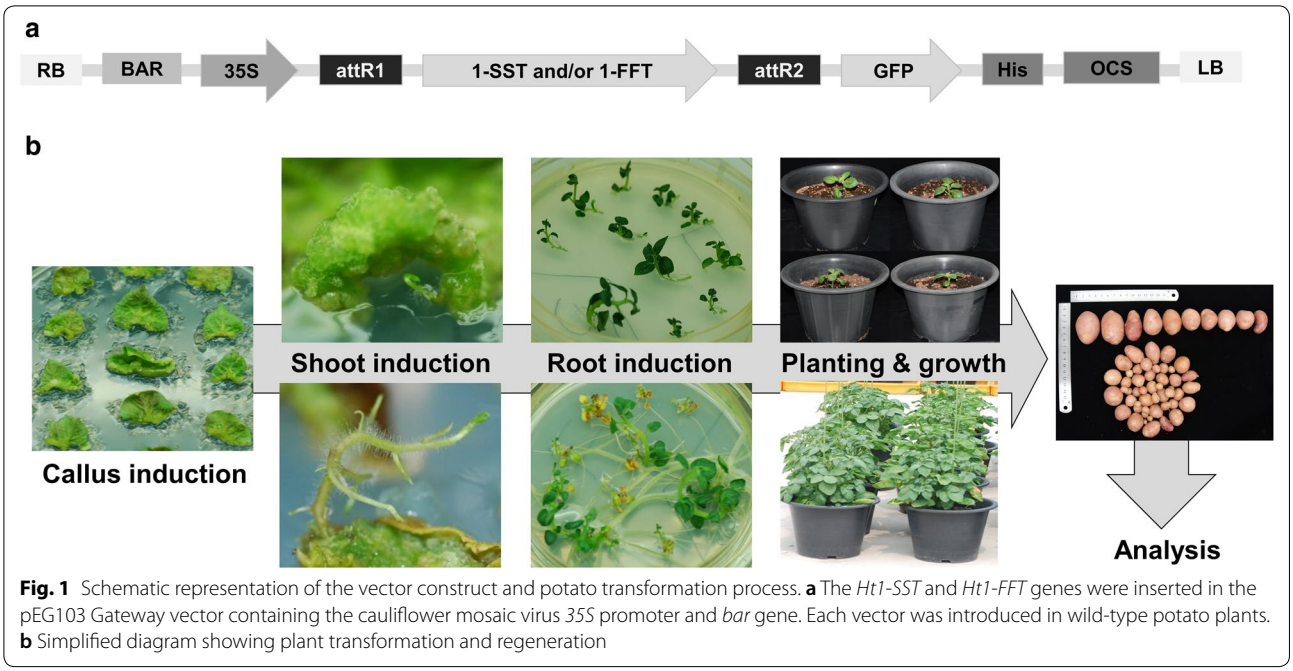

Fig. 1 Schematic representation of the vector construct and potato transformation process. **a** The *Ht1-SST* and *Ht1-FFT* genes were inserted in the pEG103 Gateway vector containing the cauliflower mosaic virus *35S* promoter and *bar* gene. Each vector was introduced in wild-type potato plants. **b** Simplified diagram showing plant transformation and regeneration

quantitative real-time PCR (qRT-PCR), and transgenic plant lines were selected.

Total RNA isolation and cDNA synthesis was performed as described previously [26]. All gene-specific primers were designed based on cDNA sequences (Table 1). Relative mRNA expression levels of *Ht1-SST* and *Ht1-FFT* were determined by qRT-PCR on a CFX Connect Real-Time PCR System (Bio-Rad, Hercules, CA) using a SYBR Green Master Mix (Enzynomics Co., Daejeon, Korea). The *actin* gene was used as a reference for data normalization. SnF transgenic plants introduced FFT-1 from transgenic plant expressing 1-SST were identified by RT-PCR with full-length 1-SST and 1-FFT primers, and SnF transgenic plant was selected.

Extraction and analysis of water-soluble carbohydrates

Five grams (fresh weight) of tissue was sampled from each harvested potato tuber using a cork borer and then homogenized in 30 ml of 20 mM sodium phosphate buffer (pH 7.0). To extract water-soluble carbohydrates, samples were incubated in a water bath at 85 °C for 30 min. After cooling to room temperature, the extracts

Table 1 Primers used for reverse transcription PCR and quantitative real-time PCR (qRT-PCR) of *fructosyltransferases* and *actin*

Gene name	GenBank accession number	Primer name	Primer sequence (5′ → 3′)	Fragment size (bp)
Actin	XM_006345899	Actin_F	GTATTGTGTTGGACTCTGGTG	614
		Actin_R	CTGCTGGAAGGTGCTGAGGGA	
		Actin_QF	GCTTCCCGATGGTCAAGTCA	101
		Actin_QR	GGATTCCAGCTGCTTCCATTC	
Ht1-SST	AJ009757	HtSST_F	CACCACATGATGGCTTCATCCACC	1893
		HtSST_R	TAACCATCAAAGTTCGAAAGTCC	
		HtSST_QF	GACCGTCGTTTTGGACCTC	294
		HtSST_QR	GCTAGAACCACCACACCAAACGGTC	
Ht1-FFT	AJ009756	HtFFT_F	GTCAGTCACCATGCAAACCC	1880
		HtFFT_R	AAAGGATAGCGATAGCCGGTA	
		HtFFT_QF	ATATCGAGGGCGTCTTATCCGGGTCTA	788
		HtFFT_QR	TCTCAAACTCTCGACTTCCTCAACGGG	

Ht1-SST, *Helianthus tuberosus* sucrose:sucrose 1-fructosyltransferase; *Ht1-FFT*, *H. tuberosus* fructan:fructan 1-fructosyltransferase

were centrifuged at 10,000×*g* and analyzed by high performance liquid chromatography (HPLC). Quantitative analysis of saccharides was performed using Sugar KS-802 column (8.0 × 300; Showa Denko K.K., Tokyo, Japan) with HPLC grade water (mobile phase) at 0.5 ml/min flow rate and 65 °C temperature on an HPLC refractive index detector (RID) system (Agilent 1100 series; Agilent Technologies, Santa Clara, CA).

Starch staining

The staining of starch in tuber tissues were performed using I_2/KI solution (2 g KI, 1 g I_2, 300 ml H_2O). Longitudinal sections of transgenic potato tubers were stained for 5 min, and washed twice with distilled water, and examined.

Statistical analysis

Data were statistically processed using Excel 2010. Error bars represent the standard deviation (SD) of three independent biological replicates. Differences between means were considered statistically significant at $p < 0.05$.

Results and discussion

The *Ht1-SST* and *Ht1-FFT* genes were introduced in potato plants via *Agrobacterium*-mediated transformation [27]. The gene isolation, vector construction, plant growth, and plant transformation methodologies are shown in Fig. 1. Among the ten antibiotic-resistant plants, two independent transgenic lines expressing either *Ht1-SST* (S4 and S5) or *Ht1-FFT* (F4 and F5) were identified by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and Reverse transcription polymerase chain reaction (RT-PCR), which were performed using both newly synthesized cDNA [26] and gene-specific primers (Fig. 2a). The SnF transgenic potato plants expressing both *Ht1-SST* and *Ht1-FFT* were obtained by the introduction of *Ht1-FFT* in the transgenic line S5, chosen for its high level of *Ht1-SST* mRNA, followed by

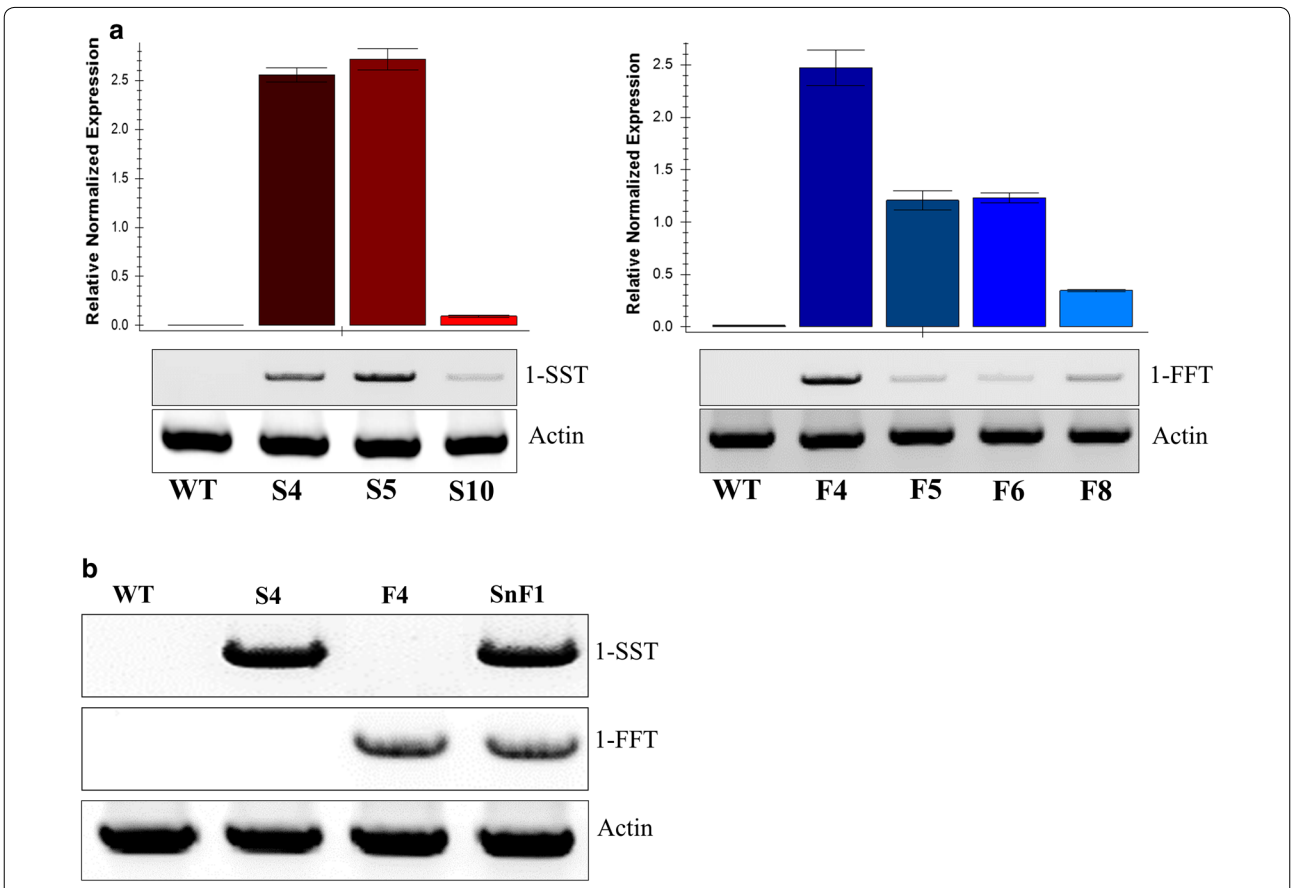

Fig. 2 Expression analysis of *Ht1-SST* and *Ht1-FFT* in transgenic potato tubers. **a** Relative expression levels of *Ht1-SST* and *Ht1-FFT* mRNAs in transgenic potato tubers using quantitative real-time PCR (qRT-PCR) and reverse transcription PCR. Expression data were normalized relative to *actin* mRNA. Melting temperatures are indicated. **b** Identification of *Ht1-SST* and *Ht1-FFT* in SnF1 transgenic potato tubers by reverse transcription PCR. WT, wild-type potato cv. Desiree (negative control); S4, S5 and S10, independent transgenic potato lines expressing *Ht1-SST*; F4, F5, F6 and F8, independent transgenic potato lines expressing *Ht1-FFT*; SnF1, transgenic potato line co-expressing *Ht1-SST* and *Ht1-FFT*

PCR-based selection of the *Ht1-FFT* gene (Fig. 2b). The phenotype, growth, and tuber size of all five independent transgenic lines (S4, S5, F4, F5, and SnF1) were compared with wild-type (WT) potato plants (Fig. 3a). Additionally, results of the starch iodine test showed no significant differences in sucrose starch metabolism among tubers of the five transgenic potato plants (Fig. 3b). These results suggest that the introduction of *Ht1-SST* and *Ht1-FFT* in potato plants has no negative impact on the growth, tuber formation, and starch biosynthesis of potato.

The *1-SST* and *1-FFT* genes encode important enzymes involved in the biosynthesis of inulin-type fructans. In this study, we performed HPLC analyses to determine the pattern of inulin-type fructans stored in transgenic potato plants expressing *Ht1-SST* and/or *Ht1-FFT* (Fig. 4). Compared with WT potato tubers, S4 and S5 potato tubers contained 3.38 ± 0.50 and 3.34 ± 0.80 mg/g DP3 inulin-type fructans, respectively (Table 2). We previously reported the measurement of inulin-type fructans from Jerusalem artichoke tubers [28]. Jerusalem artichoke tubers are composed of more than 70% fructans, so very high amounts of inulin-type fructans (approximately 693.22 mg/g) were detected, although the inulin was measured at the dry weight of the Jerusalem artichoke tuber. However, this high-expression inulin assay pattern was difficult to compare more clear results such as fructans synthesis process in inulin-type fructans analysis of transgenic potato. Therefore, we used the Jerusalem artichoke callus as a positive control, which shows relatively low inulin-type fructans synthesis compared to the Jerusalem artichoke tuber. Interestingly, DP3 inulin-type fructans accumulated to higher levels in S4 and S5 tubers than in Jerusalem artichoke callus (2.34 ± 0.82 mg/g) and SnF1 tubers (1.66 ± 0.04 mg/g). Additionally, a small amount of DP4 inulin-type fructans was detected in S4 and S5 tubers. According to Van Der Meer et al. [13], fructans in Jerusalem artichoke comprise β-(1,2)-linked fructose polymers, with a DP of up to 30. In this study, Jerusalem artichoke callus accumulated short-chain (DP3) inulin-type fructans. This finding is consistent with the observation of Pontis [29], who showed that differences in the length of fructans are not only caused by taxonomic variation but also by environmental conditions. Short-chain inulin-type fructans (DP3 and DP4) were identified in transgenic S4 and S5 potato tubers, while WT potato plants were confirmed to have no inulin-type fructans. On the other hand, no inulin-type fructans were detected in F4 or F5 tubers. These results confirmed the finding of Van Der Meer et al. [13] that 1-SST is responsible for the synthesis of DP3 inulin-type fructans from sucrose, whereas 1-FFT is unable to synthesize fructans from sucrose in the absence

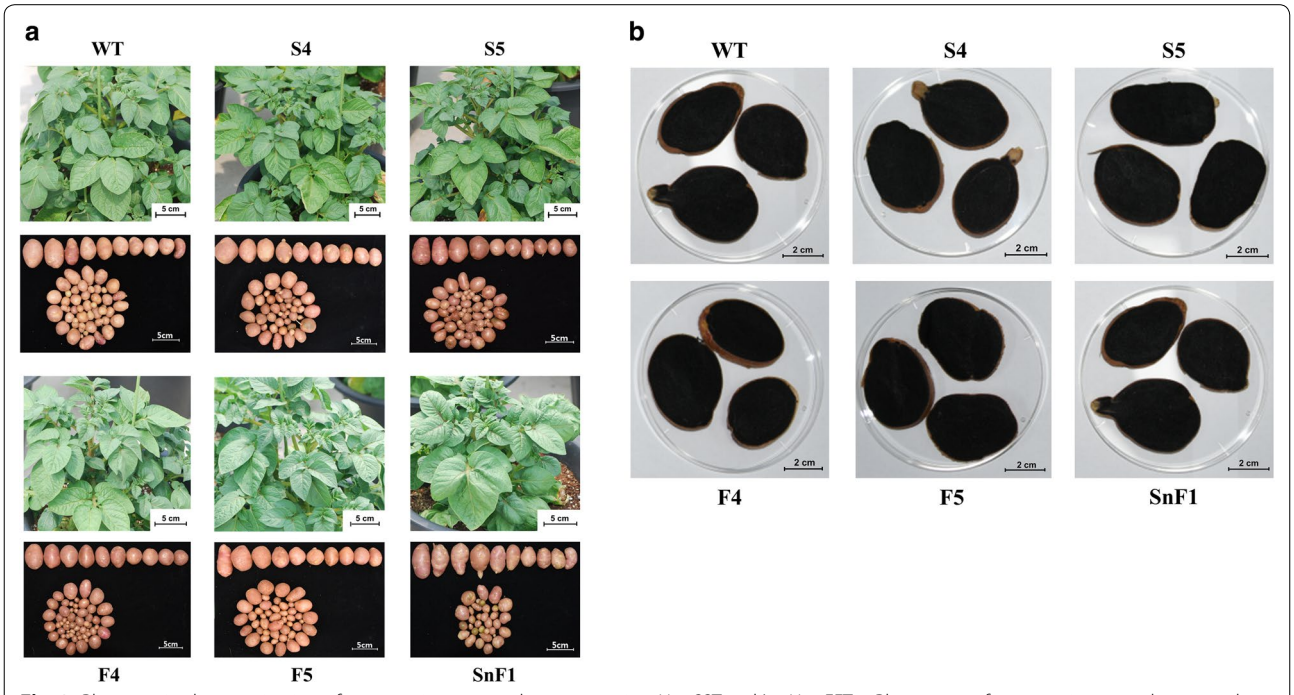

Fig. 3 Phenotypic characterization of transgenic potato tubers expressing *Ht1-SST* and/or *Ht1-FFT*. **a** Phenotype of transgenic potato leaves and tubers. **b** Analysis of starch in transgenic potato tubers using the iodine staining assay. WT, wild-type potato; S4 and S5, independent transgenic potato lines expressing *Ht1-SST*; F4 and F5, independent transgenic potato lines expressing *Ht1-FFT*; SnF1, transgenic potato line co-expressing *Ht1-SST* and *Ht1-FFT*

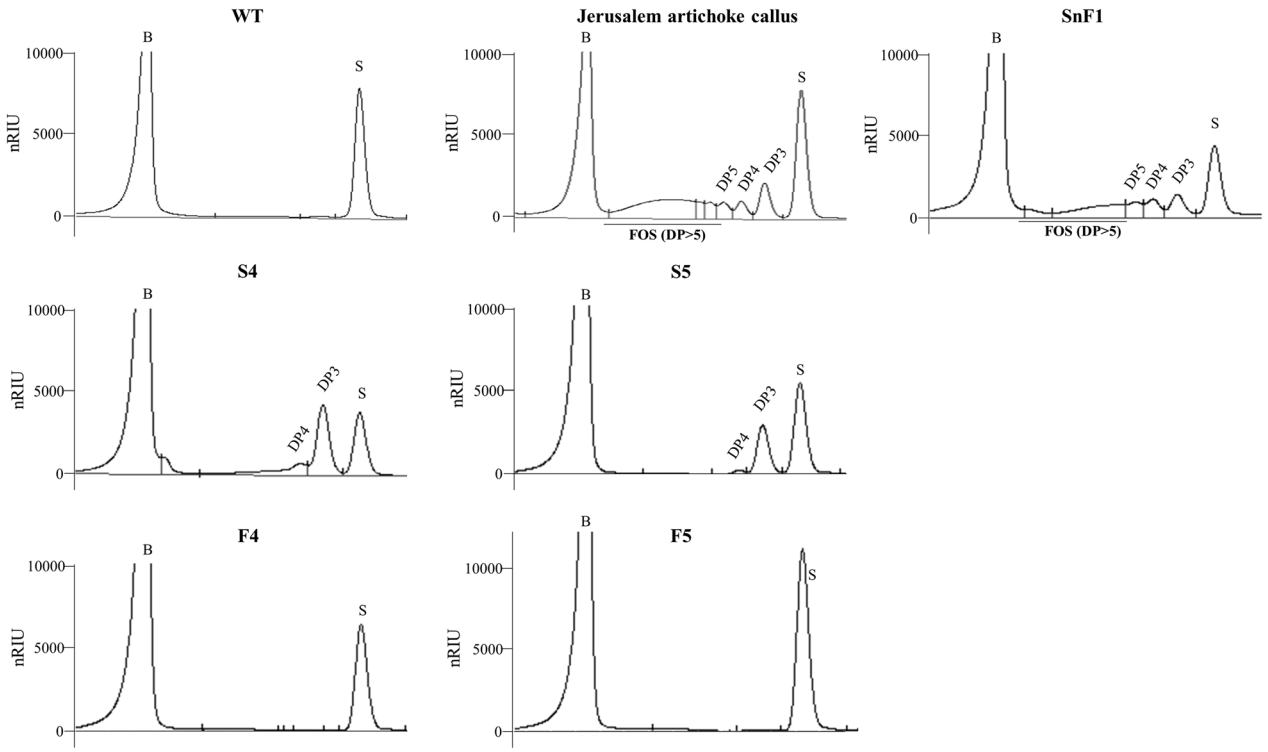

Fig. 4 HPLC analysis of soluble carbohydrates extracted from transgenic and wild-type potato tubers. B, phosphate buffer; S, sucrose; DP3, 1-kestose; WT, wild-type potato; S4 and S5, independent transgenic potato lines expressing *Ht1-SST*; F4 and F5, independent transgenic potato lines expressing *Ht1-FFT*; SnF1, transgenic potato line co-expressing *Ht1-SST* and *Ht1-FFT*

Table 2 Carbohydrate content of Jerusalem artichoke (*Helianthus tuberosus* L.) callus and transgenic potato tubers expressing *Ht1-SST* and/or *Ht1-FFT*

Plant material[a]	Gene target	Sucrose (mg/g)	Inulin-type fructans			
			1-kestose (mg/g)	DP = 4 (mg/g)	DP = 5 (mg/g)	DP > 5 (mg/g)
Wild-type potato tuber	N.A.	5.39 ± 0.37	n.d.	n.d.	n.d.	n.d
Jerusalem artichoke callus	1-SST/1-FFT	6.58 ± 0.42	2.34 ± 0.82	1.32 ± 0.28	1.14 ± 0.29	6.07 ± 2.05
S4 tuber	1-SST	1.57 ± 0.48	3.38 ± 0.50	0.29 ± 0.01	n.d.	n.d.
S5 tuber	1-SST	2.83 ± 0.13	3.34 ± 0.80	0.27 ± 0.02	n.d.	n.d.
F4 tuber	1-FFT	2.60 ± 0.10	n.d.	n.d.	n.d.	n.d.
F5 tuber	1-FFT	4.91 ± 0.09	n.d.	n.d.	n.d.	n.d.
SnF1 tuber	1-SST/1-FFT	4.75 ± 0.04	1.66 ± 0.04	0.88 ± 0.15	0.60 ± 0.15	1.11 ± 0.11

N.A. not applicable, *n.d.* not detectable, *DP* degree of polymerization

[a] S4 and S5, independent transgenic potato lines expressing *Ht1-SST*; F4 and F5, independent transgenic potato lines expressing *Ht1-FFT*; SnF1, transgenic potato line co-expressing *Ht1-SST* and *Ht1-FFT*

of 1-SST. Our results were also in agreement with previous reports of *Ht1-SST*-mediated induction of DP3 inulin-type fructans in heterologous systems, including sugar beet [30], petunia [13], and maize [17]. The SnF1 tubers accumulated short-chain inulin-type fructans to levels similar to the Jerusalem artichoke callus. Although SnF1 tubers showed a lower level of DP3 inulin-type fructans (1.66 ± 0.04 mg/g) than S4 and S5 tubers, inulin-type fructans with DP = 4 (0.88 ± 0.15 mg/g), DP = 5 (0.60 ± 0.15 mg/g), and DP > 5 (1.11 ± 0.11 mg/g) were also detected in SnF1 tubers. When fructans with DP ≥ 3 are used as a substrate, inulin-type fructans with a higher DP (> 3) are accumulated by the action of 1-FFT. This result confirms the role of 1-FFT in the production of short-chain inulin-type fructans using 1-kestose in the fructan biosynthetic pathway. Over 70% of inulin-type

fructans stored in Jerusalem artichoke tubers represent DP3–DP20 fructans [31, 32]. Species-specific differences in the DP of fructans have been described previously; for example, the DP of inulin-type fructans is higher in globe artichoke than in chicory. These differences are thought to reflect species-specific activities of 1-FFT; for example, 1-FFT derived from globe artichoke (Cs1-FFT) shows high affinity for long-chain inulin-type fructans as an acceptor substrate, whereas 1-FFT derived from chicory (Ci1-FFT) prefers short-chain (DP3) inulin-type fructans as an acceptor substrate [33, 34]. Additionally, storage conditions (prolonged storage and temperature, 18 °C, 4 °C and 4 °C under polypropylene film packing) may cause a substantial decrease in the average DP of inulin-type fructans because of depolymerization or degradation of higher molecular weight carbohydrates [35]. Depolymerization or hydrolysis of fructans is mainly caused by fructan exohydrolase, which sequentially releases fructose units from the fructan polymer, eventually leaving sucrose [36]. The 1-FFT enzyme also contributes to the de-polymerization of fructans under unfavorable conditions [36, 37]. Therefore, we speculate that the accumulation of low DP inulin-type fructans in transgenic potato tubers was caused by 1-FFT postharvest, despite its role in fructan biosynthesis during tuber formation and maturation.

Based on the activity of two enzymes, 1-SST and 1-FFT, fructan biosynthesis in another transgenic potato tuber has been previously reported. Transgenic potato tubers expressing *1-SST* alone accumulated 1.42 mg/g DP3 inulin-type fructans, whereas those co-expressing *1-SST* and *1-FFT* accumulated 2.58 mg/g DP3–DP5 inulin-type fructans [15, 17]. In this study, the S4 and S5 transgenic tubers showed an average accumulation of 3.38 and 3.34 mg/g DP3 inulin-type fructans, respectively, which is approximately 2.4-fold greater than that achieved previously (Table 2). More specifically, two independent transgenic potato lines expressing *Ht1-SST* accumulated 3.67 and 3.61 mg/g DP3 and DP4 inulin-type fructans, respectively. In SnF1 transgenic tubers, short-chain (DP3–DP5) inulin-type fructans accumulated to 3.14 mg/g, while inulin-type fructans with DP > 5 accumulated to 1.11 mg/g. Overall, SnF1 tubers accumulated a total of approximately 4.25 mg/g inulin-type fructans; however, this value was less than the total amount of inulin-type fructans accumulated in Jerusalem artichoke callus (approximately 10.87 mg/g). We also compared the level of inulin-type fructans with DP ≥ 3 between S4 and SnF1 tubers (Fig. 4). While DP3 fructans accumulated to lower levels in SnF1 tubers than in S4 tubers, the level of fructans with DP > 3 was fivefold higher in SnF1 tubers (Fig. 4). These results demonstrate that in potato, 1-FFT produces inulin-type fructans using DP3 as a substrate (synthesized by 1-SST), although 1-SST uses DP3 to produce only a small amount of DP4 fructans. Sucrose synthesized in plants is transported to amyloplasts by sucrose transporters and stored

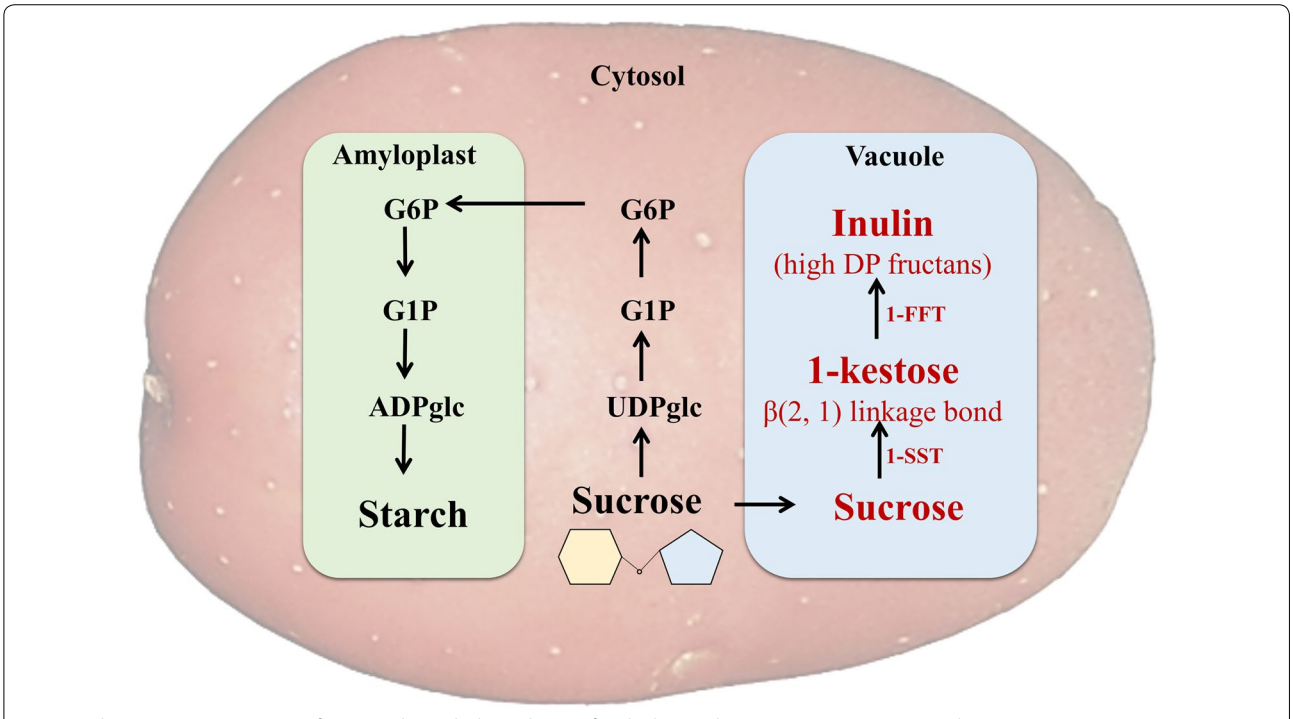

Fig. 5 Schematic representation of expected metabolic pathway of inulin biosynthesis in transgenic potato tuber

as starch. Sucrose is also transferred to vacuoles [38]. Since fructan is synthesized and stored in the vacuole, inulin could be synthesized and stored in the vacuole by *Ht1-SST* and *Ht1-FFT* in transgenic potatoes. Overall, *Ht1-SST* and *Ht1-FFT* were successfully expressed in potato, either alone or in combination, resulting in the production of inulin-type fructans to higher levels than previously achieved. A brief schematic illustrates the successful accumulation of inulin-type fructans in the vacuole and the synthesis of starch in the amyloplast in transgenic potato tubers (Fig. 5). Fructan were also detected from some of the angiosperms adapted to region having cold and dry seasons [7]. It contribute to have resistance against abiotic stress by cold [39], drought [40, 41]. The improvement of stress tolerance by triggering fructan accumulation has been demonstrated in rice [42], potato [43], tobacco [44]. These transgenic potatoes could potentially be used as a nutritional supplement with health promoting properties, and/or as a main crop capable of withstanding relatively harsh cultivation conditions.

Authors' contributions

JHJ and HSK designed and coordinated the completion of the experiments. KB and JS grew and maintained plants. H-SK and JHS performed HPLC and analyzed the data. KB, HSC, YI, HSK and JHJ analyzed the entire data. KB, HSK and JHJ wrote the manuscript. HSC and YI provided guidance and supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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