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Developmental modulation of inulin accumulation in storage organs of transgenic maize and transgenic potato

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Abstract

Many important health promoting and functional characteristics are attributed to the non-digestible polysaccharide, inulin. Its use as a prebiotic in functional food and feed has further increased inulin demand worldwide. Inulin production in crops used for food and feed application, such as maize and potato, may provide a more direct and cost-effective route to provide functional food or feed when compared to native inulin producers. Towards this end we have expressed the inulin synthesizing enzymes, 1-SST and 1-FFT from Jerusalem artichoke in maize and potato. Transgenic maize plants produced inulin type fructan (at 3.2 mg/g kernel) when expressing an endosperm targeted gene cassette. Kernel development and germination were not affected. Potato tubers expressing 1-*sst* accumulated 1.8 mg inulin/g tuber while tubers with a combined expression of 1-*sst* and 1-*fft* accumulated 2.6 mg inulin/g tuber. Inulin accumulation in maize kernels was modulated by kernel development. Inulin levels peaked and then underwent moderate degradation by late kernel development. In potato, inulin production was relatively stable throughout tuber development and little evidence of degradation was observed. The accumulation of 1-kestose in transgenic maize was positively correlated with kernel sucrose concentration. Introduction of the fructan synthetic pathway in a high-sucrose maize background increased inulin accumulation to 41 mg/g kernel. Evidence is presented indicating that sucrose availability is limiting fructan production in transgenic maize.

Keywords: Fructan; Inulin; Developmental regulation; Transgenic maize and potato

1. Introduction

Fructans are linear or branched polymers of repeating fructose residues connected by ß(2-1) and/or ß(2-6) fructosyl-fructose linkages, optionally including one terminal glucosyl unit. Fructans are widely distributed in nature occurring in bacteria, fungi and over 40,000 higher plant species [1–2]. Plants generally contain fructans with a degree of polymerization (DP) of 3–200 [2], while bacteria can produce very large fructan polymers with a DP greater than 5000 [3]. Inulin, the best characterized fructan, contains predominantly linear molecules with ß(2-1) linkages and is found in chicory (*Cichorium intybus* L.), sunflower (*Helianthus annuus* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.), among others. It is generally believed that inulin biosynthesis in plants occurs through the concerted action of two vacuolar enzymes, 1-SST (sucrose:sucrose 1-fructosyltransferase, EC 2.4.1.99) and 1-FFT (fructan:fructan 1-fructosyltransferase, EC 2.4.1.100 [4–5]. 1-SST catalyzes the conversion of sucrose to the trisaccharide 1-kestose (DP3). The elongation of 1-kestose to inulin (of DP up to 200) is catalyzed by 1-FFT. 1-SST has also been reported to produce small inulins of DP3 to DP5 [6] or DP7 [7] and thus may have some 1-FFT activity.

Many important nutritional and functional characteristics are attributed to inulin-type fructans. Inulin can increase calcium absorbance, act as a low calorie sweetener or be used as a water-soluble dietary fiber in food ingredients. Functional

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characteristics include among others taste improvement, added mouth feel, high water holding capacity and fat replacement [8–9]. More importantly, inulin is not metabolized by humans and animals and can act as a prebiotic [10]. Prebiotics enhance human and animal health performance by being selectively fermented by beneficial organisms such as *Bifidobacteria* in the large intestine of animals, at the expense of pathogenic or non-beneficial organisms such as *Salmonella*, *E. coli*, *Clostridium* species [8,9,11]. Because of this diverse range of nutritional and functional characteristics inulin is increasingly being used for improving food quality and added health benefits (functional foods) [11]. Interest in the use of prebiotics has increased among feed manufacturers, livestock and poultry producers as well [12]. Inulin type prebiotics have shown a positive effect on the health status and growth performance of Piglets [13], pets, livestock and poultry [12,14,15]. Addition of low levels (1 mg/g) of low molecular weight inulin to the diet of chicks resulted in a reduction of Salmonella colonization [16].

The major industrial sources of inulin are the chicory root and Jerusalem artichoke tuber. The commercial potential for inulin is high, however, its use is limited by the high costs of production. Transgenic crops accumulating fructan would have a significant advantage over native fructan-storing plants by making use of established breeding programs, pest resistance and adaptation to a variety of growing regions throughout the world. Transgenic approaches to produce inulin in commercial crops have been successful to a limited extent. Transgenic potato expressing the globe artichoke 1-*sst* produced the short chain inulin, 1-kestose [17]. Transgenic potato plants expressing both 1-*sst* and 1-*fft* produced inulin molecules of DP > 60, similar to the inulin profile found in the globe artichoke [18]. Transgenic sugar beet accumulated high levels of short chain inulin (DP3, DP4 and DP5) by expressing the 1-*sst* of Jerusalem artichoke [7]. The prebiotic effect of inulin, even at low doses in feed, makes its potential use in feed crops, such as corn, very attractive.

Here we report on the production of inulin and its developmental modulation in the commercially important crops maize and potato. Our data indicates that the dicot genes 1-*sst* and 1-*fft*, from Jerusalem artichoke, are fully functional in a monocot. Furthermore, the developmental modulation of inulin production in maize and potato are reported.

2. Methods

2.1. Chimeric constructs for endosperm specific expression of the Jerusalem artichoke 1-sst and/or 1-fft in transgenic maize

A construct designed for tissue specific expression of the Jerusalem artichoke 1-*sst* in maize endosperm was assembled by replacing the Cauliflower Mosaic Virus (CaMV) 35S promoter in the plasmid pSST403 [19] with a maize endosperm-specific 10 kDa zein, seed storage gene promoter [20]. The complete 1-*sst* coding sequence contained in pSST403 [19] was isolated by digesting with the restriction endonuclease enzymes NcoI and HindIII. The isolated sequence was added to the plasmid pCyt-SacB [21] containing a 10 kDa zein promoter and 3′ termination region. The maize endosperm expression cassette, containing the 10 kDa promoter, 1-*sst* coding sequence (including the native secretory and vacuole targeting signals) and the 10 kDa 3′ end, designated 10 kDa-SST, was isolated by digesting with SmaI and SalI, then ligated into the plasmid KS17. The KS17 vector contains a hygromycin resistance gene (HPT) used as the selectable marker. The final vector was designated 10 kDa-SST-17. A second construct designed for tissue specific expression of the Jerusalem artichoke 1-*fft* in maize endosperm was assembled by replacing the Cauliflower Mosaic Virus (CaMV) 35S promoter in the plasmid pSST405 [19] with a maize endosperm-specific 10 kDa zein, seed storage gene promoter [20]. The complete 1-*fft* coding sequence contained in pSST405 was isolated by digesting with NcoI and BamHI. The isolated sequence was added to the plasmid pCyt-SacB containing a 10 kDa zein promoter and 3′ termination region. PCyt-SacB was digested was NcoI and BamHI to remove the SacB region. The maize endosperm expression cassette, containing the 10 kDa promoter, 1-*fft* coding sequence (including the native secretory and vacuole targeting signals) and the 10 kDa 3′ end, designated 10 kDa-FFT**,** was isolated by digesting with SmaI and SalI, then ligated into the plasmid KS17. The final vector was designated 10 kDa-FFT-17.

2.2. Chimeric constructs for expression of the Jerusalem artichoke 1-sst and/or 1-fft in transgenic potato

For the transgenic expression of 1-*sst* and 1-*fft* from Jerusalem artichoke two binary constructs were made. The construct harboring the 1-*sst* was pSST331, the construct harboring the 1-*fft* was pFFT301 [19]. In both constructs the expression was driven by the 35S-CaMV promoter. To generate transgenic control plants a third construct was made, in which 1-*sst* from pSST331 was removed, resulting in pFB2.

2.3. Plant material and plant transformation

To generate transgenic maize plants the plant expression vector 10 kDa-SST-17 or the combined expression vectors 10 kDa-SST-17 + 10 kDa-FFT-17, and a plasmid vector encoding a selectable marker (pDetric) were co-transformed into embryogenic maize callus derived from crosses of the inbred lines A188 and B73 by micro projectile bombardment [22]. Transformed embryogenic cells were recovered on medium containing either glufosinate-ammonium or chlorsulfuron. The selectable marker pDetric contains the BAR gene, coding for phosphinothricin acetyltransferase, under the control of the 35S-CaMV promoter. A mutant acetolactate synthase gene contained in pALSLUC confers resistance to chlorsulfon. Transgenic shoots were transferred to 30 cm pots containing Metromix (Scotts–Sierra Company) media and grown to maturity in the greenhouse. Mature kernels from original transformants (first generation transformants, T1) and subsequent maize generations (T2–T4, generated through self-pollination) were grown in the greenhouse or planted directly in