Towards a better understanding of the generation of fructan structure diversity in plants: molecular and functional characterization of a sucrose:fructan 6-fructosyltransferase (6-SFT) cDNA from perennial ryegrass (Lolium perenne)

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The main storage compounds in Lolium perenne are fructans with prevailing beta(2-6) linkages. A cDNA library of L. perenne was screened using Poa secunda sucrose:fructan 6-fructosyltransferase (6-SFT) as a probe. A full-length Lp6-SFT clone was isolated as shown by heterologous expression in Pichia pastoris. High levels of Lp6-SFT transcription were found in the growth zone of elongating leaves and in mature leaf sheaths where fructans are synthesized. Upon fructan synthesis induction, Lp6-SFT transcription was high in mature leaf blades but with no concomitant accumulation of fructans. In vitro studies with the recombinant Lp6-SFT protein showed that both 1-kestotriose and 6G-kestotriose acted as fructosyl acceptors, producing 1- and 6-kestotetraose (bifurcose) and 6G,6-kestotetraose, respectively. Interestingly, bifurcose formation ceased and 6G,6-kestotetraose was formed instead, when recombinant fructan:fructan 6G-fructosyltransferase (6G-FFT) of L. perenne was introduced in the enzyme assay with sucrose and 1-kestotriose as substrates. The remarkable absence of bifurcose in L. perenne tissues might be explained by a higher affinity of 6G-FFT, as compared with 6-SFT, for 1-kestotriose, which is the first fructan formed. Surprisingly, recombinant 6-SFT from Hordeum vulgare, a plant devoid of fructans with internal glucosyl residues, also produced 6G,6-kestotetraose from sucrose and 6G-kestotriose. In the presence of recombinant L. perenne 6G-FFT, it produced 6G,6-kestotetraose from 1-kestotriose and sucrose, like L. perenne 6-SFT. Thus, we demonstrate that the two 6-SFTs have close catalytic properties and that the distinct fructans formed in L. perenne and H. vulgare can be explained by the presence of 6G-FFT activity in L. perenne and its absence in H. vulgare.
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