Title	Confirmation of fructans biosynthesized in vitro from [1-C-13]glucose in asparagus tissues using MALDI-TOF MS and ESI-MS
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Citation	Journal of Plant Physiology, 170(8), 715-722 https://doi.org/10.1016/j.jplph.2012.12.005
Issue Date	2013-05-15
Doc URL	http://hdl.handle.net/2115/53018
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Supplementary Data-1.pdf

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Supplementary Data/Material

Chemicals

[1-¹³C]glucose (99% in purity) was purchased from the Cambridge Isotope Lab. Inc. (Andover, MA, USA). Pure samples of naturally occurring (¹²C) glucose, fructose, sucrose, lactose, 1-kestose and nystose were purchased from the Wako Pure Chemical Industries Ltd. (Osaka, Japan). Naturally occurring neokestose was purified as described by Shiomi et al. (1991). Perchloric acid (60% (w/v)) was purchased from the Wako Pure Chemical Industries Ltd. Acetonitrile of HPLC grade and ethanol (synthetic, 99% in purity) was purchased from the Kanto Chemical Co. Inc. (Tokyo, Japan). 2,5-dihydroxybenzoic acid (DHB), the matrix for MALDI-TOF MS, was purchased from the Aldrich Chemical Co. Inc. (Milwaukee, WI, USA).

Details on plant material

Seeds of asparagus (*Asparagus officinalis* L. cv. Mary Washington 500W) were surface-sterilized for 20 min with sodium hypochlorite solution [containing 1% (w/v) active chlorine] that had been supplemented with 0.05% (v/v) polyoxyethylene (20) sorbitan monolaurate (Tween-20). Then they were rinsed three times with sterilized distilled water and incubated at 25 °C with 16 h of illumination daily (60 μmol m⁻² s⁻¹, from fluorescent tubes) on a medium that contained half of the nitrogen source and all other ingredients of MS medium (Murashige and Skoog, 1962) plus 0.7% (w/v) agar (pH adjusted to 5.7), but no sucrose (seeds have starch as carbohydrate reserve). The medium was dispensed into glass bottles (8 cm in diameter and 12 cm in height) and then autoclaved for 15 min at 120 °C (1.1 kg cm⁻²) before use. Segments of stems (0.5-0.7 mm in diameter without the cladophyll) of

approximately 10 mm in length were prepared from the resultant 12-week-old seedlings and used as plant material for experiments.