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Drought induces fructan synthesis and 1-SST (sucrose: sucrose fructosyltransferase) in roots and leaves of chicory seedlings (*Cichorium intybus* L.)

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Abstract. Seeds of *Cichorium intybus* L. var. *foliosum* cv. Flash were sown in acid-washed vermiculite and grown in a controlled-environment growth chamber. After 1 month of growth, plantlets did not contain sucrose:sucrose 1-fructosyltransferase (1-SST), the key enzyme in fructan biosynthesis. No fructan could be observed. Some of the plants were submitted to drought for 2 weeks. Glucose, fructose and sucrose concentrations increased in roots and leaves of stressed plants and the fructan concentration in roots and leaves was ten times higher than in control plants. The onset of fructan synthesis coincided with the increase in 1-SST activity in roots. Expression of the 1-SST gene could be observed in roots and leaves of stressed plants.

Key words: *Cichorium* (drought stress) – Drought – Gene expression (drought induced) – Fructan – Sucrose

Introduction

Fructan is a major reserve carbohydrate in some economically important plant orders such as the Poales, Liliales and Asterales (Meier and Reid 1982; Nelson and Spollen 1987). Different fructan types can be distinguished depending on the type of glycosidic linkage: inulin consists of $\beta(2 \rightarrow 1)$ linkages, levan of $\beta(2 \rightarrow 6)$ linkages and graminan of both $\beta(2 \rightarrow 1)$ and $\beta(2 \rightarrow 6)$ linkages. The roots of *Cichorium intybus* predominantly accumulate fructan of the inulin type up to a degree of polymerisation (DP) of 55. The enzyme generally considered to initiate fructan synthesis (Edelman and Jefford 1968; Lüscher et al. 1996; Van den Ende and Van Laere 1996) is sucrose:sucrose 1-fructosyltransferase (1-SST; EC 2.4.1.99; Glc-Fru + Glc-Fru → Glc-Fru-Fru + Glc, where Glc = glucose and Fru = fructose) which catalyses fructosyltransfer from one sucrose (Suc) molecule to another resulting in Glc and 1-kestose formation. Chain elongation is made possible by fructan:fructan 1-fructosyltransferase (EC 2.4.1.100; $Glc\text{-}Fru_n + Glc\text{-}Fru_m \Leftrightarrow Glc\text{-}Fru_{(n-1)} + Glc\text{-}Fru_{(m+1)}$, with $n > 1$, $m > 0$), which catalyses fructosyltransfer from one fructan molecule to another. Fructan 1-exohydrolase (EC 3.2.1.80; $Glc\text{-}Fru_n + H_2O \Rightarrow Glc\text{-}Fru_{(n-1)} + Fru$, with $n > 1$) is responsible for fructan degradation.

Fructan can either be accumulated both as a short- or long-term reserve carbohydrate in heterotrophic organs such as roots, stems and grains or in autotrophic organs such as leaves (Pollock 1986; Pollock and Cairns 1991). Besides the well-known role of fructan as a reserve carbohydrate, many other physiological functions have been proposed. Fructan concentrations increase under anoxia (Albrecht et al. 1993), low temperature (Jeong and Housley 1990; Prud'homme et al. 1993), high CO_2 concentrations (Smart et al. 1994) or continuous illumination of excised leaves (Simmen et al. 1993; Penson and Cairns 1994). Fructan remobilization plays a role during rapid inflorescence development (Bieleski 1993; Solhaug and Aares 1994).

Until now the effect of drought on the concentration of fructan and the activity of fructosyltransferases has mainly been studied with grasses. Depolymerisation of fructan has been observed in tall fescue under drought stress (Spollen and Nelson 1994). Other reports, however, suggest that, during drought, tolerant genotypes accumulate higher levels of fructan than the more sensitive ones (Volaire and Lelièvre 1997; Kerepesi et al. 1998). In the leafy liverwort *Porella platyphylla*, drought led to an accumulation of high-molecular-weight fructan (Marschall et al. 1998). Transgenic fructan-accumulating tobacco exhibited enhanced resistance to drought stress (Pilon-Smits et al. 1995). A role for fructan in drought resistance was also suggested by Hendry (1993), who stated that (i) the appearance of fructan-producing

Abbreviations: DP = degree of polymerisation; Fru = fructose; Glc = glucose; RM = relative μmol; 1-SST = sucrose:sucrose 1-fructosyltransferase; Suc = sucrose

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taxa (30–15 million years ago) corresponds with a climatological shift towards seasonal drought and (ii) that the distribution of present-day fructan flora corresponds with regions of seasonal drought.

In this manuscript we investigated whether drought stress could induce fructan synthesis in young chicory plants not yet accumulating fructan. Therefore, we studied fructan concentrations, 1-SST enzymatic activities and 1-SST mRNA concentrations in both leaves and roots of stressed and control plants.

Materials and methods

Plant material and growth conditions. *Cichorium intybus* L. var. *foliosum* cv. Flash (Hortiplan, Roeselare, Belgium) seeds were sown in acid-washed vermiculite (2.5 l) in perforated plastic boxes in a growth chamber under the following conditions: 16 h of light (Philips TLD 36 W/84; photosynthetically active radiation 350 μmol m^{-2} s^{-1}) at 20 °C and 8 h of darkness at 15 °C. Each box contained 28 plantlets. The seeds received a nutrient solution (250 ml d^{-1}) containing 2 mM NH_4NO_3, 1 mM $MgSO_4$, 1 mM K_2HPO_4, 71.7 μM NaFeEDTA, 2 mM $CaCl_2$, 12 μM $MnSO_4$, 46 μM H_3BO_3, 145 nM $(NH_4)_6Mo_7O_{24}$, 1.53 μM $ZnSO_4$, and 1.56 μM $CuSO_4$. Four weeks after sowing (i.e. day 0), half of the plants were deprived of nutrient solution. The drought process was followed by measuring the water content of the vermiculite. Therefore, a sample of 30 g vermiculite was taken and dried for several days at 60 °C where upon the dry weight was obtained.

Sampling. Samples for carbohydrate analysis and enzymatic activities were taken at day 0, 1, 2, 5, 7, 8, 12 and 13 after drought start. At each sampling date, 3 to 10 plantlets (yielding at least 0.8 g of root material) per treatment were harvested in triplicate. The vermiculite was carefully removed from the roots. Both leaf and root fresh weights were measured, and dry weights were determined.

Tissue extraction and carbohydrate analysis. A defined amount (x g; $x \geq 0.8$) of root and leaf tissue was homogenized using a mortar and pestle in an equal amount (x ml) of extraction buffer as described in Van den Ende et al. (1998). About 500 mg of tissue homogenate was diluted twice with 0.02% (w/v) Na-azide and put in a water bath at 95 °C for 15 min. Further treatment and analysis were as described by Van den Ende et al. (1998). Quantification was performed using mannitol as an internal standard. Conversion factors (relative to mannitol) for Glc, Fru, Suc, 1-kestose and 1,1-nystose were obtained by using the external-standards method. Since the higher oligofructans (DP > 4) were not available, their response coefficients were estimated using the work of Chatterton et al. (1993). Therefore, the fructan concentrations shown in the figures are given as "RM" (i.e. relative μmol, because the real response coefficients of DP > 4 on Dionex were not experimentally determined) instead of μmol.

Protein extraction and assay of 1-SST. The original homogenate was centrifuged for 5 min at 10 000 *g*. Duplicate samples (300 μl) of the supernatant were mixed with 1.2 ml of saturated $(NH_4)_2SO_4$ [final saturation 80% $(NH_4)_2SO_4$]. After 0.5 h of incubation on ice, precipitates were collected by centrifugation at 10 000*g* for 5 min. Subsequently, the precipitates were washed by vortexing in 1 ml of 80% $(NH_4)_2SO_4$ (pH 5.7) and treated further as described by Van den Ende et al. (1998). Protein concentrations were assayed by the method of Lowry et al. (1951). The activity of 1-SST was measured and analysed as described by Van den Ende et al. (1999). Activity is expressed in units (U) per g FW. One unit of 1-SST activity is defined as the amount of enzyme which produces 1 μmol of 1-kestose in 1 min.

Statistics. Values on the graphs represent means of three replicates. The corresponding SE values are indicated.

Preparation of chicory 1-SST and 18S ribosomal probes. Based on the 1-SST cDNA sequence of industrial chicory (De Halleux and Van Cutsem 1997), two oligonucleotide primers were chosen (5′ATGGCTTCCTCTACCACC3′ and 5′CGTTGCCAGTGTAGAGCAT3′) and used during one-step reverse transcriptase-polymerase chain reaction (RT-PCR; PCR Access; Promega) on total chicory RNA (RNeasy Plant Mini Kit; Qiagen). The RT reaction was performed at 48 °C for 45 min. The PCR conditions were as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min. Final extension was at 72 °C during 10 min. The resulting PCR fragment was subsequently subcloned in a TOPO-XL vector according to the manufacturer's descriptions (TOPO-XL PCR Cloning Kit; Invitrogen) and several clones were sequenced using M13 forward and reverse primers on an automatic DNA-sequencing apparatus using a dye-terminator cycle-sequencing kit (ABI-PRISM; Eurogentec). One partial 1-SST cDNA clone was subsequently used as a template for preparing PCR product which was labelled by a random-primed method using the DNA-labelling T7 QuickPrime Kit (Pharmacia Biotech) and [α-^{32}P]dCTP as described by Feinberg and Vogelstein (1984).

By using the same techniques as described for the 1-SST probe, a partial 18S rRNA probe (400 bp) from chicory was prepared by RT-PCR, subcloned and labelled. Two rRNA-specific primers (5′AGACTGTGAAACTGCGAATGG3′ and 5′ TTGTCACTACCTCCCCGTGT) derived from *Tagetes* (Accession U42501) were used and the annealing temperature during PCR was 60 °C.

Extraction and Northern-blot hybridisation of RNA. Samples of RNA were taken at every sampling date from both control and stressed plants. Total RNA from 0.5 g of frozen roots and leaves was extracted using an RNA extraction kit (TR-118; Euromedex). The RNA (10 μg) was denatured in 50% formamide, 17% formaldehyde and 10% Mops buffer [200 mM Mops, (pH 7.0), 50 mM Na-acetate, 1 mM EDTA] at 65 °C for 5 min and fractionated on a 1.2% agarose gel containing 2.2 M formaldehyde in Mops buffer. The RNA was transferred to a Hybond-NX membrane (Amersham) in 200 mM NaH_2PO_4 (pH 7.5) containing 3.6 M NaCl and 20 mM EDTA. The bound RNA was crossed-linked using a GS gene linker (BioRad) and was kept at 80 °C for 2 h. After a 2-h prehybridization [250 mM Na_2HPO_4 (pH 7.3), 7% SDS, 1% BSA], hybridization with 1-SST probe was carried out for 18 h at 65 °C in a buffer identical to the prehybridization solution. After hybridisation, four washes (total time: 1.5 h) were done in 20 mM Na_2HPO_4 (pH 7.3) containing 1% SDS at 65 °C. The washed membrane was exposed to a BAS-MP intensifying screen and was made visible using a PhosphorImager BAS 1000 Fujix. To ensure that equal amounts of RNA were loaded in each well, the membrane was also hybridized with radiolabelled 18S rRNA probe from chicory.

Results

Growth characteristics. The water content of the vermiculite was measured to give an overall idea of the drought rate. Under control conditions, the water content of the vermiculite remained more or less constant. A roughly linear loss in moisture was observed under stress conditions (data not shown). Because we wanted to start drought at a very young stage before fructan synthesis starts in control plants, the roots were very small and contained thin hairy side roots which were difficult to fully retrieve from the vermiculite. Throughout the drought experiment, both leaf and root fresh weights were lower under stress conditions than

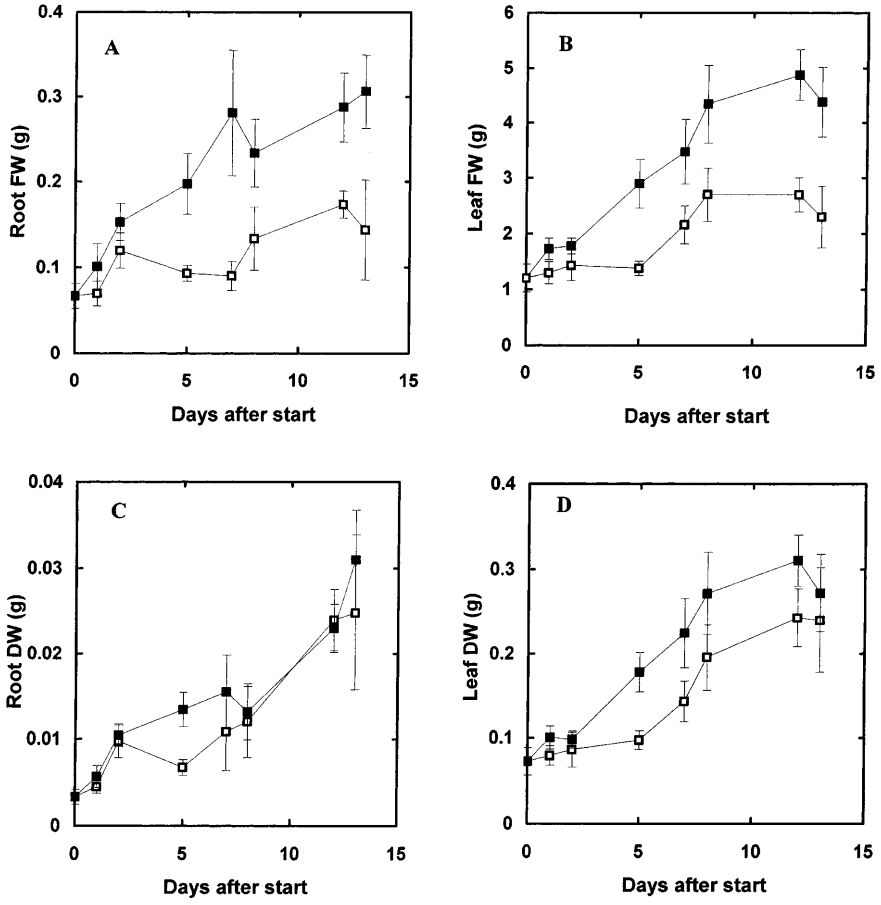

Fig. 1A–D. Changes in root fresh weight (**A**), leaf fresh weight (**B**), root dry weight (**C**), and leaf dry weight (**D**) of 3–10 young chicory plantlets under control (■) and drought-stress (□) conditions. Means ± SE are presented

under control conditions (Fig. 1A,B). However, no significant difference was found on a dry-weight basis (Fig. 1C,D). After up to 12 d of drought, stressed leaves remained turgescent and even appeared to have a darker green colour than control leaves. However, from day 13 on, stressed leaves rapidly turned brownish and lost all turgescence (data not shown).

Changes in carbohydrate concentration in roots and leaves. The concentration of Suc in the roots of stressed plants remained unchanged during the first 2 d of drought (Fig. 2C). Thereafter, the Suc concentration gradually increased from 3.7 to 22.3 μmol (g FW)$^{-1}$ over the next 10 d. The Suc concentration in the control plants increased slightly during the experiment. At the end of drought, the Suc concentration in stressed plants was ~3-fold higher than in control plants. The Glc (Fig. 2A) and Fru (Fig. 2B) concentrations varied only slightly in the roots of control plants whereas under stress conditions there was an ~5-fold concentration increase. In leaves, just as in root tissue, no changes in Glc (Fig. 2D), Fru (Fig. 2E) and Suc (Fig. 2F) concentrations could be observed during the first 2 d of drought. The concentrations of Glc, Fru and Suc in the control plantlets remained constant throughout the experiment. The Suc concentration in leaves from stressed plants increased and at the end of drought reached a concentration twice as high as in the control plants (Fig. 2F). The Glc and Fru concentrations fluctuated more but remained higher than in the control plants (Fig. 2D,E).

Under normal growth conditions, leaves of *Cichorium intybus* only accumulate a negligible amount of 1-kestose and no higher-DP fructan can be found in leaves of field-grown *Cichorium intybus* throughout the growing season (data not shown). At day 0, the 4-week-old plantlets of *Cichorium intybus* only contained Glc, Fru and Suc (Fig. 2), and no fructan could be observed in the roots (Fig. 3A). Two days after the start of drought, the fructan concentration increased in the roots of stressed plantlets and a 10-fold higher concentration was reached at the end of the experiment compared to control plantlets (Fig. 3A). After 13 d of drought, the fructan DP in roots of stressed plants was 50 whereas in control plants a maximum DP of 32 could be observed (data not shown). After 5 d of drought, the fructan concentration in leaves increased (Fig. 3B) and fructans up to a DP of 28 were observed (data not shown). The final fructan concentration in leaves of stressed plants was 3 RM (g FW)$^{-1}$ [estimated at 30 mg (g DW)$^{-1}$; Fig. 3B] whereas in roots the concentration was up to 83 RM (g FW)$^{-1}$ [estimated at 735 mg (g DW)$^{-1}$; Fig. 3A].

Changes in the activity and expression of 1-SST. The 1-SST activity in roots of control plants remained very low and increased only slightly on day 7 (Fig. 4A). In roots of stressed plants, however, a sharp increase in 1-SST activity could be observed starting from day 2. The

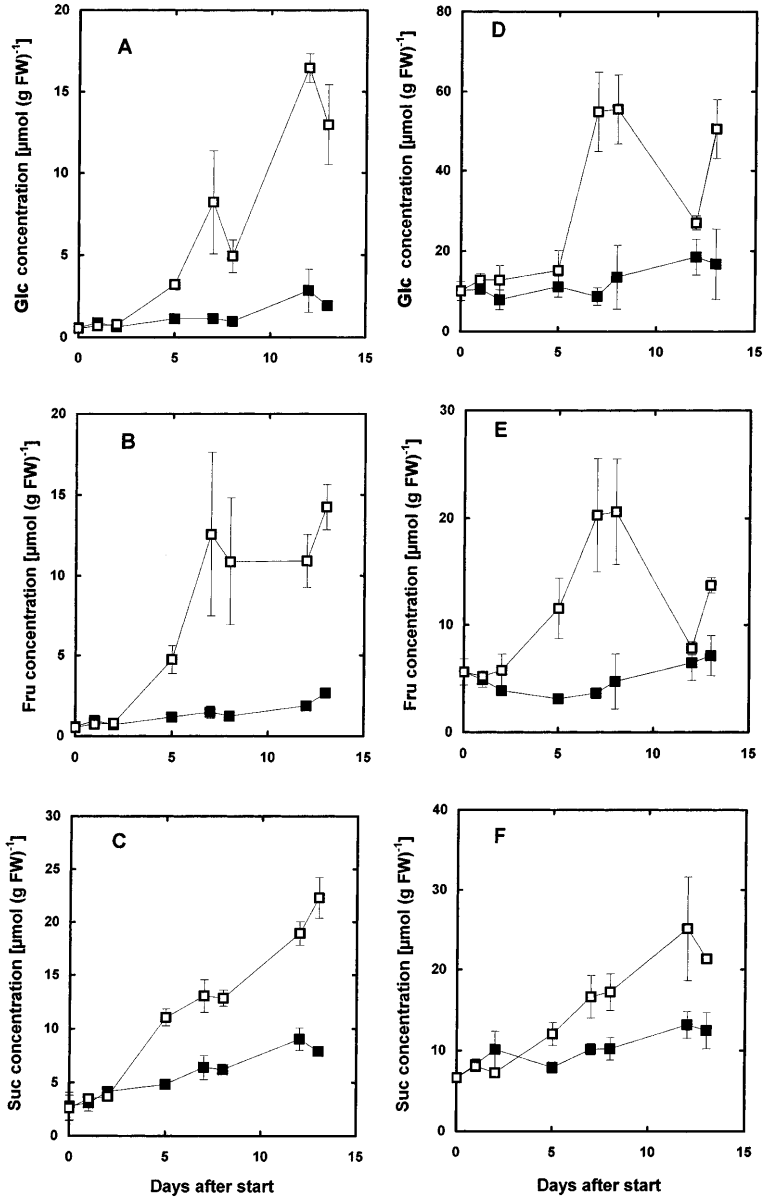

Fig. 2A–F. Concentrations of Glc (**A, D**) Fru (**B, E**) and Suc (**C, F**) in young chicory roots (**A, B, C**) and leaves (**D, E, F**) of control plants (■) and of plants subjected to drought (□)

1-SST activity decreased between day 5 and day 12, but activities remained higher than in control plants. No expression of 1-SST in roots and leaves could be observed at day 0 by measuring mRNA (Fig. 4B). At day 2, the appearance of a faint band could be observed in roots of stressed plants. From day 5 onwards, intense expression of 1-SST occurred in the roots of desiccated plants (Fig. 4B) but not in the roots of control plants. At the last sampling date 1-SST expression diminished. Control leaves (Fig. 4B) showed no detectable expression of 1-SST. In stressed plants, however, expression started at day 7. In general, 1-SST was expressed more abundantly in roots than in leaves.

Discussion

Fructans and fructan-metabolizing activities could not be detected in 4-week-old chicory seedlings grown under non-stress conditions. Therefore, these seedlings are excellent for studying if fructan metabolism can be initiated by several external factors, which can be varied in a controlled growth chamber. We focused our attention on drought stress since the relation between fructan metabolism and drought stress is not straightforward (see *Introduction*). Since it is clear that 1-SST is the key enzyme in starting fructan biosynthesis (Van den Ende et al. 1996), we concentrated our efforts on the induction of the 1-SST gene in relation to 1-SST activities and fructan concentrations. The growth rate of stressed plants was limited especially on a fresh-weight basis (Fig. 1) with a loss of turgescence toward the end of the drought period. Similar to previously reported drought-stress data (Kerepesi et al. 1998; Van den Ende et al. 1998), an increase in Glc, Fru and Suc concentrations could be observed in roots (Fig. 2A–C) and leaves (Fig. 2D–F) under drought stress. The Suc concentration increased to approximately the same

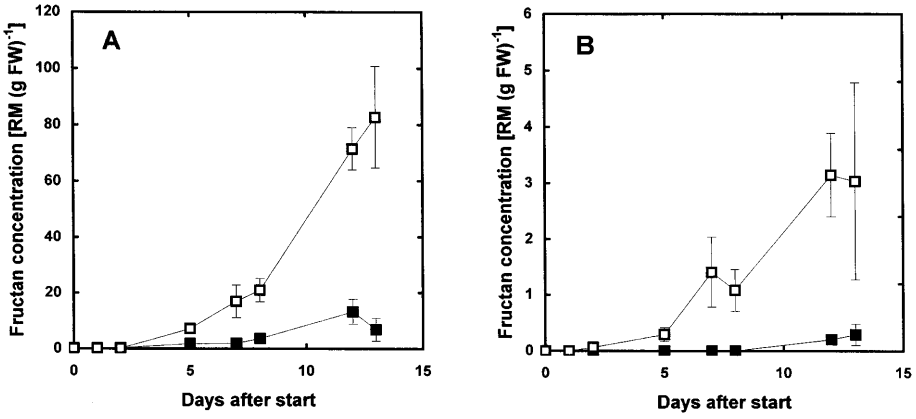

Fig. 3A,B. Fructan concentrations in roots (**A**) and leaves (**B**) of control chicory plants (■) and plants submitted to drought (□)

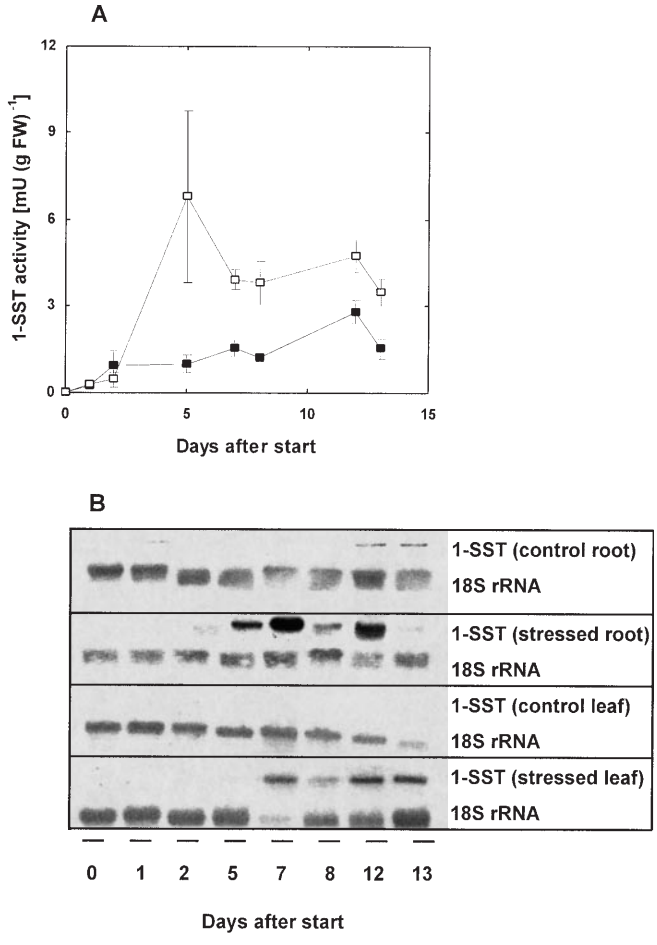

Fig. 4. **A** Activity of 1-SST in young roots of control chicory plants (■) and of plants submitted to drought (□). **B** Expression of 1-SST in roots and leaves of control plants and desiccated plants. Each section consists of the expression of 1-SST, followed by the expression of an 18S rRNA probe

concentration in roots and leaves, i.e. 25 μmol (g FW)$^{-1}$. The enhanced Suc concentrations can easily be explained by the fact that growth is limited to a much higher extent than photosynthesis. Moreover, it is well known that drought stress is accompanied by a shift in the partitioning of photosynthate in favour of sucrose synthesis (Hare et al. 1998). This is probably achieved by the activation of sucrose phosphate synthase through reversible protein phosphorylation (Toroser and Huber 1997). The increased hexose and Suc concentrations agree with the overall observation that environmental stress leads to accumulation of organic compounds (saccharides and polyhydric alcohols) and zwitterionic alkylamines (amino acids and quaternary ammonium compounds) in many plants. In particular, sugar increase occurs in a wide range of plants grown at low moisture level (Bohnert et al. 1995).

Exposure to drought resulted in the accumulation of large amounts of fructan in the roots of young plantlets of *Cichorium intybus* (Fig. 3A). Under normal conditions no fructans are found in chicory leaves. However, it was possible to induce fructan synthesis in leaves (Fig. 3B), albeit to a much lower extent than in roots. This is consistent with a previous report (Sprenger et al. 1997) where fructans were initiated in detached chicory leaves upon incubation in Suc. In the leafy liverwort *Porella platyphylla* desiccation also led to an increase in the fructan pool (Marschall et al. 1998) and field-grown *Vernonia herbacea* exhibit an increase in total fructan concentration during winter when cerrado plants are exposed to temperature and drought stress (Machado de Carvalho and Dietrich 1993).

A strong increase in 1-SST activity in desiccated roots could be observed from day 5 (Fig. 4A), which correlates well with the expression of the 1-SST gene (Fig. 4B) and the subsequent onset of fructan synthesis (Fig. 3A). At the last sampling date, fructan concentration was still high (Fig. 3A), but 1-SST activity (Fig. 4A) and 1-SST mRNA concentrations (Fig. 4B) decreased again, probably due to continuing dehydration of the roots finally leading to plant death. Sucrose may not only play a role as a substrate for 1-SST, but from these and other results (Sprenger et al. 1997; Vijn et al. 1998; Van den Ende et al. 1999) we strongly suggest that Suc may act as an effector inducing the 1-SST gene. The 1-SST activity seems to increase when the Suc concentration becomes twice as high as on day 0 (Fig. 2C,F), suggesting that there is a certain threshold Suc concentration for 1-SST induction (Pollock and Cairns 1991). In the roots and stubble of ryegrass, a positive correlation has been found between Suc concentration and 1-SST activity (Prud'homme et al. 1992). However, no correlation could be

found in the leaves of ryegrass and barley (Prud'homme et al. 1992; Wang and Tillberg 1996).

It was demonstrated here that drought, probably in an indirect way via increased Suc concentrations, can induce fructan synthesis in plants not yet accumulating fructan. Until now, no clear proof has been presented that fructan in situ can act as drought protectant. Generally, it can be assumed that the accumulation of osmolytes and thus of water-soluble carbohydrates is associated with osmoregulation, although until now no conclusive link has been demonstrated between osmoregulation and stress tolerance (Hare et al. 1998). The ability to maintain membrane integrity and prevent protein denaturation under drought stress appears to be one of the factors linked to dehydration tolerance since subcellular structures must be maintained in an aqueous environment. Recently, bacterial fructans with a high DP have been shown to cause a large increase in the surface pressure of lipid monolayers at the air-water interface. This indicates a profound and specific membrane interaction of the fructans which may prevent lipid condensation and phase transitions from taking place (Demel et al. 1998). This may suggest that the interaction of fructans in vivo with membranes is responsible for water-stress protection.

In conclusion, growth limitation by drought stress resulted in an increase in Glc, Fru and Suc concentration in roots and leaves of young chicory plants. As a result of drought, the 1-SST gene was induced, 1-SST enzymatic activities increased and fructans accumulated to a much higher extent compared to non-stressed plants.

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References

Albrecht G, Kammerer S, Praznik W, Wiedenroth EM (1993) Fructan content of wheat seedlings (*Triticum aestivum* L.) under hypoxia and following re-aeration. New Phytol 123: 471–476

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell 7: 1099–1111

Bieleski RL (1993) Fructan hydrolysis drives petal expansion in the ephemeral daylily flower. Plant Physiol 103: 213–219

Chatterton NJ, Harrison PA, Thornley WR, Benett JH (1993) Separation and quantification of fructan (inulin) oligomers by anion exchange chromatography. In: Fuchs A (ed) Inulin and inulin-containing crops. Elsevier, Amsterdam, pp 93–99

De Halleux S, Van Cutsem P (1997) Cloning and sequencing of the 1-SST cDNA from chicory root (Accession No. U81520) (PGR97-036). Plant Physiol 113: 1003–1003

Demel RA, Dorrepaal E, Ebskamp MJM, Smeekens JCM, De Kruijff B (1998) Fructans interact strongly with model membranes. Biochim Biophys Acta 1375: 36–42

Edelman J, Jefford TG (1968) The mechanism of fructosan metabolism in higher plants as exemplified in *Helianthus tuberosus*. New Phytol 67: 517–531

Feinberg AP, Vogelstein B (1984) Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137: 266

Hare PD, Cress WA, Van Staden J (1998) Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21: 535–553

Hendry GAF (1993) Evolutionary origins and natural functions of fructans – a climatological, biogeographic and mechanistic appraisal. New Phytol 123: 3–14

Jeong B, Housley TL (1990) Fructan metabolism in wheat in alternating warm and cold temperatures. Plant Physiol 93: 902–906

Kerepesi I, Galiba G, Bányai E (1998) Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedlings. J Agric Food Chem 46: 5347–5354

Lowry OH, Rosebrough NJ, Farr AL, Randall RH (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275

Lüscher M, Erdin C, Sprenger N, Hochstrasser U, Boller T, Wiemken A (1996) Inulin synthesis by a combination of purified fructosyltransferases from tubers of *Helianthus tuberosus*. FEBS Lett 385: 39–42

Machado de Carvalho MA, Dietrich SM (1993) Variation in fructan content in the underground organs of *Vernonia herbacea* (Vell.) Rusby at different phenological phases. New Phytol 123: 735–740

Marschall M, Proctor MCF, Smirnoff N (1998) Carbohydrate composition and invertase activity of the leafy liverwort *Porella platyphylla*. New Phytol 138: 343–353

Meier H, Reid JSG (1982) Reserve polysaccharides other than starch in higher plants. In: Loewus FA, Tanner W (eds) Encyclopedia of plant physiology (NS), vol 13A. Plant carbohydrates. Springer, Berlin, pp 418–471

Nelson CJ, Spollen WG (1987) Fructans. Plant Physiol 71: 512–516

Penson SP, Cairns AJ (1994) Fructan biosynthesis in excised leaves of wheat (*Triticum aestivum* L.): a comparison of the de novo synthesis in vivo and in vitro. New Phytol 128: 395–402

Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek PJ, Smeekens SCM (1995) Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol 107: 125–130

Pollock CJ (1986) Fructans and the metabolism of sucrose in vascular plants. New Phytol 104: 1–24

Pollock CJ, Cairns AJ (1991) Fructan metabolism in grasses and cereals. Annu Rev Plant Physiol Plant Mol Biol 42: 77–101

Prud'homme MP, Gonzalez B, Billard JP, Boucaud J (1992) Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of ryegrass (*Lolium perenne* L.) as affected by source/sink modification after cutting. J Plant Physiol 140: 282–291

Prud'homme MP, Gastal F, Belanger G, Boucaud J (1993) Temperature effects on partitioning of ^{14}C assimilates in tall fescue (*Festuca arundinacea* Schreb.). New Phytol 123: 255–261

Simmen U, Obenland D, Boller T, Wiemken A (1993) Fructan synthesis in excised barley leaves. Plant Physiol 101: 459–468

Smart DR, Chatterton NJ, Bugbee B (1994) The influence of elevated CO_2 on non-structural carbohydrate distribution and fructan accumulation in wheat canopies. Plant Cell Environ 17: 435–442

Solhaug KA, Aares E (1994) Remobilization of fructans in *Phippsia algida* during rapid inflorescence development. Physiol Plant 91: 219–225

Spollen WG, Nelson CJ (1994) Response of fructan to water deficit in growing leaves of tall fescue. Plant Physiol 106: 329–336

Sprenger N, Schellenbaum L, Van Dun K, Boller T, Wiemken A (1997) Fructan synthesis in transgenic tobacco and chicory plants expressing barley sucrose: fructan 6-fructosyltransferase. FEBS Lett 400: 355–358

Toroser D, Huber SC (1997) Protein phosphorylation as a mechanism for osmotic-stress activation of sucrose-phosphate synthase in spinach leaves. Plant Physiol 114: 947–955

Van den Ende W, Van Laere A (1996) De-novo synthesis of fructans from sucrose in vitro by a combination of two purified

enzymes (sucrose:sucrose fructosyl transferase and fructan:fructan fructosyl transferase) from chicory roots (*Cichorium intybus* L.). Planta 200: 335–342

Van den Ende W, Van Wonterghem D, Verhaert P, Dewil E, De Loof A, Van Laere A (1996) Purification and characterization of 1-SST, the key enzyme initiating fructan biosynthesis in young chicory roots (*Cichorium intybus* L.). Physiol Plant 98: 455–466

Van den Ende W, Moors S, Van Hoenacker G, Van Laere A (1998) Effect of osmolytes on the fructan pattern in feeder roots produced during forcing of chicory (*Cichorium intybus* L.). J Plant Physiol 153: 290–298

Van den Ende W, De Roover J, Van Laere A (1999) Effect of nitrogen concentration on fructan and fructan metabolizing enzymes in young chicory plants (*Cichorium intybus*). Physiol Plant 105: 2–8

Vijn I, Van Dijken A, Lüscher M, Bos A, Smeets E, Weisbeek P, Wiemken A, Smeekens S (1998) Cloning of sucrose:sucrose 1-fructosyltransferase from onion and synthesis of structurally defined molecules from sucrose. Plant Physiol 117: 1507–1513

Volaire F, Lelièvre F (1997) Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought stress in the south of France. Aust J Agric Res 48: 933–944

Wang C, Tillberg JE (1996) Effect of nitrogen deficiency on accumulation of fructan and fructan metabolizing enzyme activities in sink and source leaves of barley (*Hordeum vulgare*). Physiol Plant 97: 339–345