Journal of Plant Physiology **165** (2008) 1572—1581

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ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

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Low temperature and defoliation affect fructan-metabolizing enzymes in different regions of the rhizophores of *Vernonia herbacea*

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Received 8 October 2007; received in revised form 21 January 2008; accepted 22 January 2008

KEYWORDS
Brazilian Cerrado;
Defoliation;
Fructans;
Low temperature;
Tolerance to stress

Summary

In addition to the storage function, fructans in Asteraceae from floras with seasonal growth have been associated with drought and freezing tolerance. *Vernonia herbacea*, native of the Brazilian Cerrado, bears underground reserve organs, rhizophores, accumulating inulin-type fructans. The rhizophore is a cauline branched system with positive geotropic growth, with the apex (distal region) presenting younger tissues; sprouting of new shoots occurs by development of buds located on the opposite end (proximal region). Plants induced to sprouting by excision of the aerial organs present increased 1-fructan exohydrolase (1-FEH) activity in the proximal region, while plants at the vegetative stage present high 1-sucrose:sucrose fructosyltransferase (1-SST) in the distal region. The aim of the present study was to analyze how low temperature (5 °C) could affect fructan-metabolizing enzymes and fructan composition in the different regions of the rhizophores of intact and excised plants. 1-SST and 1-fructan:fructan fructosyltransferase (1-FFT) were higher in the distal region decreasing towards the proximal region in intact plants at the vegetative phase, and were drastically diminished when cold and/or excision were imposed. In contrast, 1-FEH increased in the proximal region of treated plants, mainly in excised plants subjected to cold. The ratio fructo-oligo to fructo-polysaccharides was significantly higher in plants exposed to low temperature (1.17 in intact plants and 1.64 in excised plants) than in plants exposed to natural temperature conditions (0.84 in intact vegetative plants and 0.58 in excised plants), suggesting that oligosaccharides are involved in the tolerance of plants to low temperature via 1-FEH, in addition to 1-FFT. Principal component analysis indicated

Abbreviations: DP, degree of polymerization; 1-FEH, 1-fructan exohydrolase; 1-FFT, 1-fructan:fructan fructosyltransferase; GPC, gel-permeation chromatography; HPAEC/PAD, high-performance anion exchange chromatography; M_r, relative molecular mass; PCA, principal components analysis; 1-SST, 1-sucrose:sucrose fructosyltransferase.

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0176-1617/$ - see front matter
doi:10.1016/j.jplph.2008.01.004

different response mechanisms in fructan metabolism under defoliation and low temperature, which could be interpreted as part of the strategies to undergo unfavorable environmental conditions prevailing in the Cerrado during winter.

Introduction

Most plants store starch or sucrose as reserve carbohydrates, but about 15% of all flowering plant species store fructans, which are linear and branched polymers of fructose (Hendry and Wallace, 1993). Unlike starch, fructans are water-soluble compounds stored in the vacuoles (Wiemken et al., 1986), although their exclusive vacuolar localization has been argued, as the presence of fructans and fructan mobilization enzymes was reported in the apoplastic fluid of crown tissues of oat (Livingston and Henson, 1998) and wheat (Van den Ende et al., 2005).

Among the plants that store fructans are many of significant economic importance and highly evolved families, including Asteraceae (Hendry and Wallace, 1993). *Cichorium intybus*, *Helianthus tuberosus*, *Taraxacum officinale*, *Viguiera discolor* and *Vernonia herbacea*, among other Asteraceae, store high amounts of inulin-type fructans with linearly $\beta(2\rightarrow1)$-linked fructo-furanosyl units (Carvalho et al., 2007). Inulin is synthesized by the combined action of at least two different fructosyltransferases (Edelman and Jefford, 1968; Koops and Jonker, 1996; Van den Ende and Van Laere, 1996): 1-sucrose:sucrose fructosyltransferase (1-SST) catalyzing the formation of 1-kestose from two molecules of sucrose with the release of glucose and 1-fructan:fructan fructosyltransferase (1-FFT) mediating the reversible transfer of fructosyl units between inulins of different chain length. Inulin depolymerization occurs by the action of 1-fructan exohydrolase (1-FEH) via the hydrolytic cleavage of terminal fructosyl residues (Van Laere and Van den Ende, 2002). Fructan storage is induced at high sucrose concentration (Pollock and Cairns, 1991), whereas the breakdown occurs for reserve mobilization when energy supply is needed, e.g. after defoliation (Morvan-Bertrand et al., 2001; Van den Ende et al., 2001; Asega and Carvalho, 2004) or to increase oligofructan concentrations under stress (Dias-Tagliacozzo et al., 1999; Van Laere and Van den Ende, 2002).

The size of the fructosyl polymers deposited in storage organs varies between species and according to developmental stage and environmental factors (Pollock et al., 1996; Van den Ende et al., 2002; Carvalho et al., 2007), although a wide range of chain lengths occurs simultaneously in the fructan pool of a same tissue (Carvalho and Dietrich, 1993). The variation in chain length of the inulin polymers in different Asteraceae species could be the result of different enzymatic characteristics of 1-FFT (Hellwege et al., 1998; Vergauwen et al., 2003) and a consequence of differences in the fructan exohydrolase activity that defines species-specific sets of inulins and are active not only during fructan mobilization but also in developing reserve organs (Marx et al., 1997; Itaya et al., 2002, 2007). Since fructan composition can vary according to developmental stage and environmental factors, time-specific expression of different enzymes or isoenzymes could also be responsible (Hellwege et al., 1998). De Roover et al. (1999) demonstrated that defoliation caused a rapid increase in 1-FEH II expression in roots, suggesting that it may be induced as a survival enzyme when energy demands increase and fast re-growth is necessary.

Fructans have often been reported to play physiological roles other than carbon storage, such as the lowering of sucrose concentration in the cell and thus the prevention of sugar-induced feedback inhibition of photosynthesis (Pollock et al., 1996). Fructans might also contribute to protect plants against water deficit caused by drought or low temperatures (Pilon-Smits et al., 1995; Livingston and Henson, 1998) possibly through the stabilization of membranes under these stressing conditions (Demel et al., 1998; Hincha et al., 2007). The apoplastic localization of fructans (Livingston and Henson, 1998; Van den Ende et al., 2005) provides good evidence that these carbohydrates confer stability to cell membranes exposed to freezing temperatures.

The presence of fructans in Asteraceae from the Brazilian Cerrado has been well documented (Tertuliano and Figueiredo-Ribeiro, 1993) and associated with the seasonal growth exhibited by the Cerrado flora as well as with the drought and low temperatures prevailing in winter (Carvalho et al., 2007). Variations in fructan content and composition and in fructan-metabolizing enzymes have been reported during the phenological cycle of *V. herbacea* (Carvalho and Dietrich, 1993; Portes and Carvalho, 2006), and fructan mobilization by 1-FEH concomitant with a decline in 1-SST activity was