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FRUCTANS FROM ELONGATING LEAF BASES ARE A SOURCE OF CARBON FOR REGROWTH AFTER DEFOLIATION IN *LOLIUM PERENNE*

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Abstract

A detailed study of carbohydrate metabolism in perennial ryegrass (*Lolium perenne* L.) during the first 48 h of regrowth showed that the decline in fructan concentration occurred not only in the differentiation zone (30-60 mm from leaf base), but also in the elongation zone of the elongating leaf bases. Unlike other soluble carbohydrates, the net deposition rate of fructose remained positive and even rose during the first day following defoliation. FEH (fructan exohydrolase) activity, which was maximum in the differentiation zone before defoliation, increased in all segments but peaked in the elongation zone after defoliation. Taken all together, these data strongly suggest that fructans stored in the leaf growth zone were hydrolysed and recycled in that zone to sustain leaf growth, i.e. the restoration of active photosynthesis, immediately after defoliation.

Keywords: carbohydrates, defoliation, elongating leaf bases, fructans, ryegrass.

Acronyms: FEH, fructan exohydrolase; SST, sucrose : sucrose fructosyltransferase.

Introduction

When defoliation involves the removal of all the photosynthetically active tissues, the C supply to leaf meristem depends transiently on carbon reserves. Leaf sheaths are generally considered as the major storage site since they can accumulate up to 70 % of the fructans stored in the vegetative parts of grasses (Volenec, 1986; Morvan *et al.*, 1997). Even if

elongating leaf bases act as strong sinks towards imported C assimilates, they also synthesize fructans in subsequent amounts (Schnyder and Nelson, 1987). The main objectives of this study were to evaluate the fate of fructans stored in elongating leaf bases and to assess their contribution to leaf growth. To this end, we focused our study to the first hours following defoliation and we combined this small time scale with the recognition of the function differences that exist along the axis of the elongating leaves.

Material and Methods

Seeds of *Lolium perenne* L. cv. Bravo were germinated and grown for eight weeks in controlled environment on a nutrient solution as described previously by Morvan-Bertrand *et al.* (1999a). After 0, 5, 24 and 48 h of regrowth, plants were harvested and elongating leaf bases (0 to 60 mm from leaf base) were dissected longitudinally into 10 mm-long fragments.

Carbohydrates were extracted as described by Morvan-Bertrand *et al.* (1999a). Carbohydrates were separated and quantified by high-performance liquid chromatography (HPLC) on a Sugar-PAK column (300x6.5 mm, Millipore waters, USA), eluted with 0.1 mM CaEDTA in water.

Plant tissue was ground in 50 mM citrate-phosphate buffer (pH 5.7) containing 5 mM dithiotreitiol (DTT). After filtration through Miracloth, the homogenate was centrifuged at 20,000 g for 10 min. An aliquot of the supernatant was desalted on Sephadex G50, which also allowed the elimination of sucrose from the extract. The assay mixture consisted of 100 µL enzyme extract and 100 µL of substrate. For FEH (fructan exohydrolase) activity, high-molecular weight fructan (8 $mg.mL^{-1}$) extracted from *Lolium perenne* were used as substrate. For SST (sucrose : sucrose fructosyltransferase) activity assay, 100 mM sucrose was used as substrate. After incubation at 30 °C for 2 h (SST) or 4 h (FEH), 100 µL mannitol (1 $g.L^{-1}$) was added to the assay mixture. Fructose and 1-kestose in the assay mixture were quantified by HPLC on the Sugar-PAK column under the conditions defined above for WSC analysis.

Results and Discussion

Effect of defoliation on the spatial distribution of WSC concentration in elongating leaf bases

Before defoliation, fructan concentration was high in the basal 20 mm (Fig.1A). Sucrose concentration was maximal at leaf base and decreased gradually along the leaf axis (Fig.1B). In contrast, monosaccharide concentrations were very low in the first 10 mm of the leaf, increased markedly throughout the elongation zone and reached a maximum at about 30 mm (glucose) and 45 mm (fructose) above the leaf base (Fig.1, C and D). Apart from fructose concentration, which remained roughly constant in the growing zone (division and elongation zones) during the first 5 hours of regrowth, concentrations of other carbohydrate fractions decreased during that period. Decline in fructan concentration was much more important in the growing zone than in the differentiation zone. The concentration of all carbohydrates decreased between 5 and 24 hours after defoliation to reach a very low level along the whole leaf axis.

The local rates of sugar deposition indicate the rate to which carbohydrates are deposited in order to maintain the observed sugar concentrations while tissue is growing (Fig.1E-H). In the first 5 hours after defoliation, the net deposition rate of total WSC decreased sharply and became strongly negative in all leaf segments, indicating that soluble carbohydrates were used much faster than they were imported and/or synthesised. This decline was attributable to the decrease of fructan, sucrose and glucose depositions (Fig.1E-G). In contrast, a positive net deposition of fructose was observed in the fast growing zone during the first hours of regrowth, suggesting that the deposited fructose (Fig.1H) came at least partly from the fructan breakdown. Between 24 h and 48 h after defoliation, deposition rates of the carbohydrates reached zero in all segments of the leaf axis suggesting that, on the second day of regrowth, import of carbohydrates was restored to balance their use in that tissue.

Effect of defoliation on enzyme activities

Before defoliation, FEH activity was low in the basal segment and increased gradually with distance from leaf base (Fig.2A). SST activity was highest in the first segment where fructan deposition was the most important (Fig.2B). It declined progressively throughout the growing zone, and remained at a low level in the differentiation zone. After 24 hours of regrowth, FEH activity had increased in all segments. The maximum value, which was located in the most distal segment before defoliation (50-60 mm), occured next to the growing

zone after defoliation. During the second day of regrowth, it shifted in the elongation zone (20-30 mm). SST activity declined by 50 % in the elongation zone (0-30 mm) during the first day of regrowth and remained at a low level in the differentiation zone.

Post-defoliation use of fructans accumulated in the leaf growth zone

As in several grasses and cereals (Schnyder and Nelson, 1987; Roth *et al.*, 1997), fructans from perennial ryegrass are synthesised and accumulate in the leaf growth zone. This is suggested by the high fructan concentration and the high SST activity found in the proximal segment of the elongating leaves. The low fructan concentration as well as the high FEH and the low SST activities in the differentiation zone suggest that cells mobilised their fructan reserves when they are displaced away from the meristem and moved through the differentiation zone, where the products of their hydrolysis might be used for secondary cell wall deposition (Allard and Nelson, 1991).

After defoliation, one could wonder if fructans are hydrolysed only in the differentiation zone, as it happens before cutting, or if they are also hydrolysed locally, *i.e.* in the cell division and elongation zones. The results presented here provide three arguments in favour of the latter hypothesis. First, the decline in fructan concentration occurred, not only in the differentiation zone but also in the cell elongation zone. Second, unlike all other soluble carbohydrates, the net deposition rate of fructose remained positive and even rose during the first day following defoliation. This indicates that fructose probably coming from fructan breakdown was transiently generated at a higher rate than it was used. Third, FEH maximum activity, which was located in the differentiation zone before defoliation, shifted in the cell elongation zone thereafter. Taken together, these observations strongly suggest that fructans stored in the leaf growth zone were hydrolysed and recycled in the cell division and elongation zones. This finding is of particular interest for an understanding of defoliation tolerance and grassland species persistence. Refoliation, i.e. restoration of active photosynthesis, is the crucial element of the plant's response to severe defoliation, reducing the time of dependence to stored C resources and allowing a rapid transition to current photosynthate (Richards, 1993; Morvan-Bertrand *et al.,* 1999b). Utilisation of carbohydrates which were already present in the leaf growth zone at the time of defoliation, could be

considered as one of the mechanisms that may facilitate a rapid refoliation, because investment of C from other sources is reduced.

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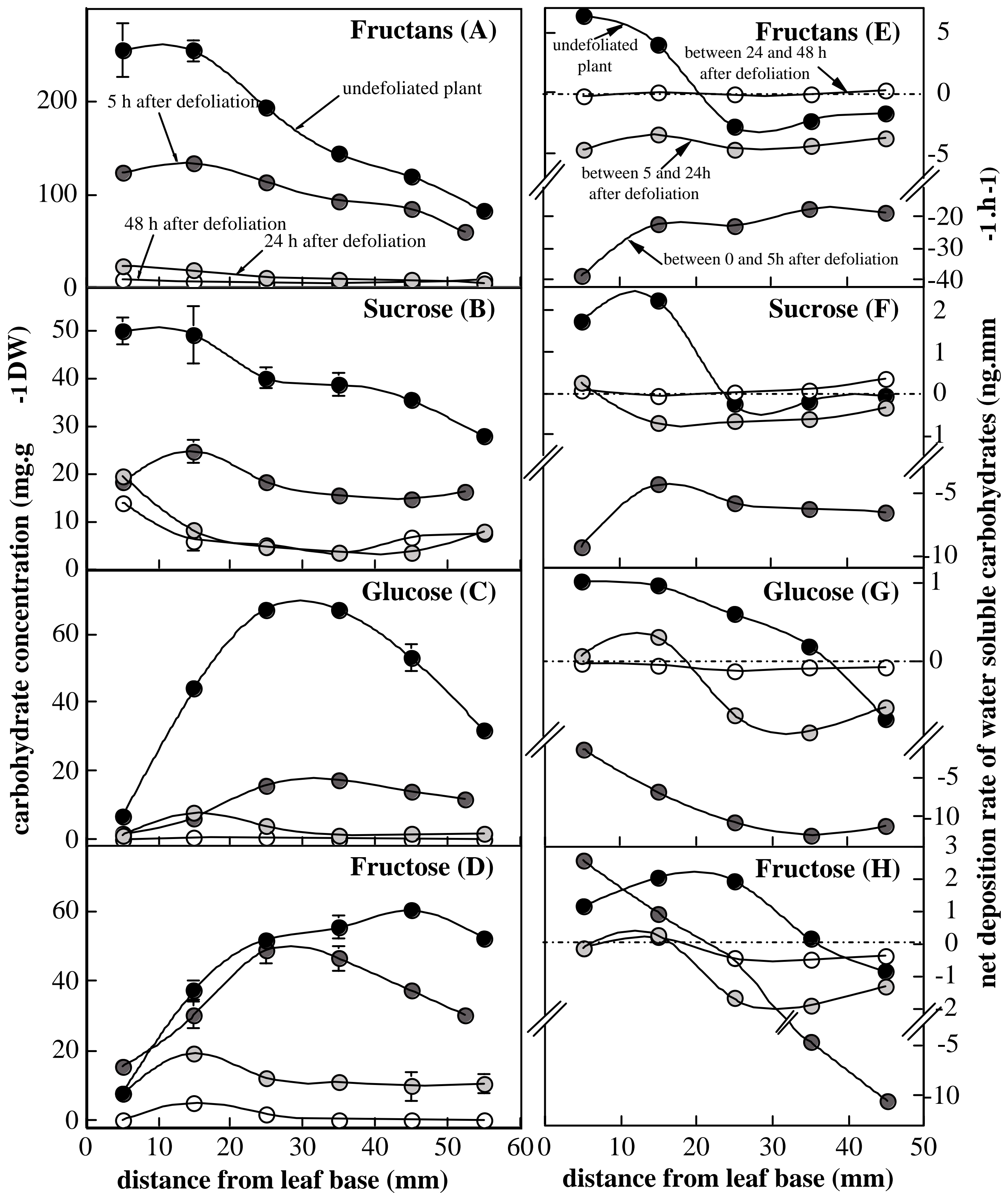

Figure 1 - Changes in spatial distribution of carbohydrate concentrations (A-D) and net deposition rate of carbohydrates (E-G) in elongating leaf bases of *Lolium perenne* at the time of defoliation (0 h) and after 5 h, 24 h, and 48 h of regrowth. Values are means of three replicates. Vertical bars indicate ± SE when larger than the symbol.

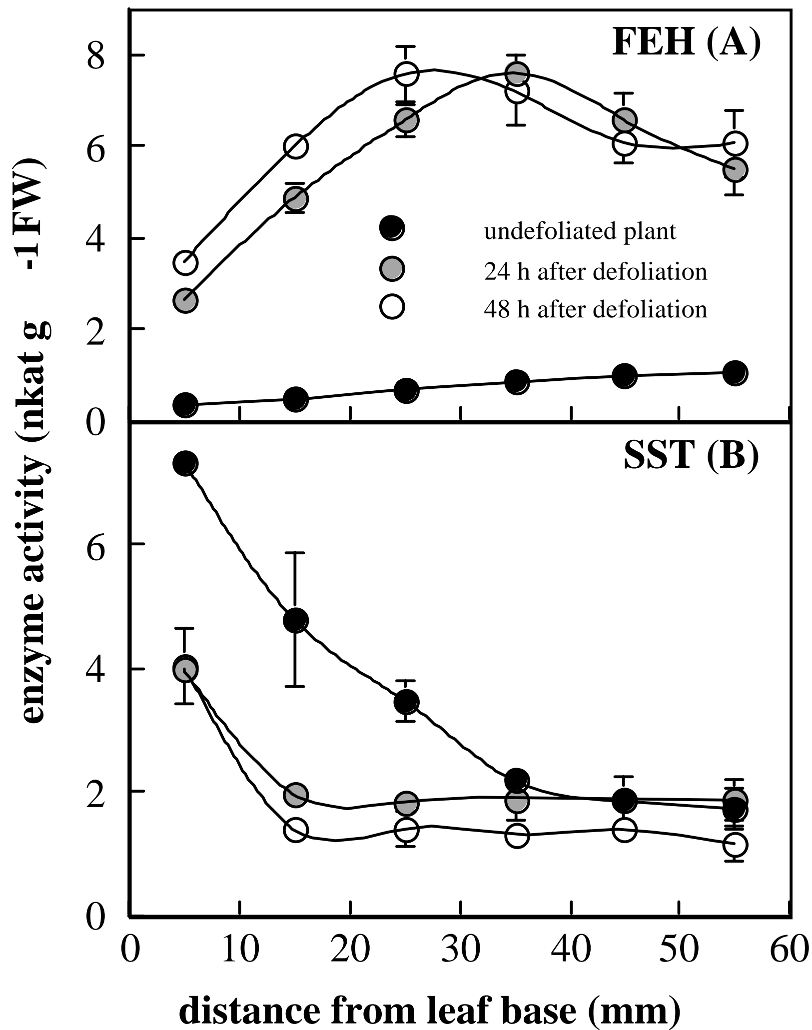

Figure 2 - Changes in spatial distribution of FEH (A) and SST (B) activities in elongating leaf bases of *Lolium perenne* at the time of defoliation (0 h) and after 24 h and 48 h of regrowth. Values are means of three replicates. Vertical bars indicate ± SE when larger than the symbol.