AN OVERVIEW OF THE PHYSIOLOGY AND BIOCHEMISTRY OF N RESERVES MOBILIZATION IN FORAGE SPECIES

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ABSTRACT

Recent works focusing on the physiological and biochemical events associated to perennial forages sustainability (re-growth after defoliation, winter survival), provided evidence that vegetative storage proteins (VSPs, ca proteins stored in remaining organs and specifically mobilized) were key organic compounds for shoot re-growth. Attempts to elucidate environmental conditions involved in VSPs deposit ability of different species are under progress today. In contrast, mechanisms of spring- or cut-induced proteolysis are investigated at a lesser extend. This contribution is an opportunity to summarize our knowledge of N-reserves mobilization and to set the question of VSPs breakdown regulation.

KEYWORDS

Defoliation, nitrogen, proteolysis, re-growth, reserves, vegetative storage proteins.

INTRODUCTION

Perennial forage species are subjected both to winter survival and to successive defoliation / re-growth cycles resulting from agricultural practices such as cutting or grazing. It is well established that moderate to severe clipping, as well as a decline in foliar growth in autumn, result in a transient reduction of photosynthesis and growth-related nitrogen assimilation. The differentiation of new tissues, during early spring growth or post-harvest re-growth, is initiated by mobilization of organic reserves, previously accumulated in perennial storage organs. Since the pioneering investigations of Graber *et al*. (1927), putting forward the evidence that shoot re-growth of defoliated alfalfa was associated with a rapid decline in taproot total nonstructural carbohydrates, the widely admitted view is in favour of a predominant contribution of sugar reserve to forage plant persistence ability. This current view has been clearly reassessed during the last few years, and sustained by recent studies providing evidence for a physiological and biochemical participation of endogenous N pools, mainly represented by proteins.

Whatever the nature of the reserves involved (C or N), one should keep in mind that a *quantitatively significant mobilization* refers to polymer degradation, and is achieved by an enhancement of enzymatic activity expressions associated with macromolecules hydrolysis in the preliminary stage following defoliation. This metabolic change is an absolute pre-requisite to the necessary exchange between a static pool (stored) and a xylem allocable pool of plant constituents, and allows to define reserve availability rather than reserve quantity. According to the admitted wisdom mentioned above, hydrolysis of carbohydrate polymers has been much more investigated than protein degradation.

The aim of this paper is to resume our knowledge on the physiology and biochemistry of N reserve mobilization in forage plant species, with a special interest taken to the regulation of peptidase expression. A better understanding of the regulation of mobilization processes when plants resume growth in spring or immediately after clipping is essential to appreciate how forage plants recover from these disturbances.

Metabolic responses of forage plants impaired by loss of leaf material Short-term effects resulting from loss of foliar tissues following clipping are represented by two main physiological disorders. Schematically (Fig. 1), removal of most photosynthetic area reduces strongly the supply of photoassimilates to storage organs, and induces a preferential allocation of carbon to growing tissues (i.e. meristematic zones and intact young leaves). In the meantime, ammonium as well as nitrate net uptake are reduced (Ourry *et al*., 1990). The mechanisms leading to this lower activity of root N uptake systems remain unknown, but it can be hypothesized that, among other factors, increase in amino acids pool resulting from protein hydrolysis may reduce these N transporters. Therefore, regrowth is largely initiated at the expense of previously stored organic reserves including N compounds such as soluble proteins. Recent studies using 13C and 15N labeling (Avice *et al*., 1996a) provided evidence that a significant amount of C and N in growing tissues of alfalfa were derived from N reserves, while more than 60% of stored carbohydrates were used for root respiration and only a small proportion (5%) of stored C to re-growing shoots. This suggests a compensatory mechanism in energy and reducing power supplies during a lag period of heterotrophic conditions. Although it is not known whether respiration rate in remaining tissues is affected by shoot removal, it is clear that origin of substrates is modified (Fig. 1).

In a second period, development of newly active tissues ensures an increase of mineral nitrogen uptake leading to progressive replenishment of N reserve pools (amino acids and proteins), higher photosynthesis allowing restoration of carbohydrate reserves in storage organs, and a direct allocation of recently fixed C to sustain respiration (Fig. 1).

N reserves within the plant. Because high cellular concentrations of amino acids and nitrate would lead to osmotic imbalance in plants, protein-N constitutes the largest pool of nitrogen in plant cells. According to numerous studies, amino-N is the most readily used N pool present in storage organs, while protein-N represents the largest quantity of stored N in perennial forage plants (see Volenec *et al*., 1996 for a review). During the last decade, special interest has been taken in peculiar proteins specifically involved in storage function (Staswick, 1988), and defined as to vegetative storage proteins (VSPs). In forages, VSPs have been characterized in alfalfa taproot and stolons of white clover, and their contribution to shoot spring growth (Hendershot & Volenec, 1993a; Bouchart *et al*., 1997) or post-harvest re-growth (Hendershot & Volenec, 1993b; Corre *et al*., 1996) demonstrated.

Works focusing on seasonal variations of VSPs deposit, and recent characterization of genes encoding for VSP synthesis, give evidence that factors controlling VSPs accumulation are under progress. In contrast, little is known about the molecular structure of VSPs that point them to preferential proteolysis.

Mechanisms for proteins breakdown. Mobilization of nitrogen from storage organs results from increased peptisase activity (Ourry *et al*., 1989; Gordon *et al*., 1990; Hendershot & Volenec, 1993a; Desjouis *et al*., 1996) before translocation of amino acids and amides to the active growing sites. The subcellular distribution of alfalfa VSPs has recently been investigated by immunolocalization (Avice *et al*., 1996b). The presence of VSPs in the vacuolar compartment, widely designed as the "lytic" compartment of plant cells, associated to their coexistence with short-life proteins, implies that the proteolytic machinery can specifically recognize VSPs as substrates during N mobilization induction. One can suggest that several distinct proteolytic pathways exist, and that specific features of VSPs (molecular mass, pI, cleavage bonds,…) order recognition by peptidases. In addition, it should be noted that mobilizations of C and N reserves follow the same pattern, suggesting the occurrence of common regulatory mechanism at the molecular level. Nevertheless, a specific regulation of peptidase expression cannot

be excluded in order to explain the cyclic pattern of synthesis / degradation of VSPs.

Regulation of protease activities. Very little is known about the identification of factors controlling protein degradation, especially in forages. During the last decade, the mobilization of macromolecules has largely been studied through the regulation mechanisms of hydrolytic enzymes expressed in cells or organs submitted to nutrient starvation, and some promotors and inhibitors of hydrolases have been identified. Among such regulators, the role of polyamines (PAs) have been partly ascribed to their ability to regulate proteolytic (Kaur-Sawhney *et al*., 1982) or amylasic (Sung *et al*., 1994), activities. The molecular mechanisms by which PAs may regulate enzymatic activities have been mainly attributed to electrovalent linkages between polycationic amines and negatively charged groups of enzymes or their respective substrates. Evidence for a PAs-mediated control of protein mobilization during post-clipping re-growth has recently been established in white clover (Desjouis *et al*., 1996). For the first 6 d following defoliation, the increase in endopeptidase expression was associated to a 80% decline in free PAs in storage organs. These changes, supported by the fact that PAs may inhibit partially endopeptidase expression *in vivo* and *in vitro* suggests that the decline in free PAs content in storage organs during the first period of re-growth would permit the expression of proteolytic activity and thus trigger the hydrolysis of N reserves.

CONCLUSION

It is now obvious that N-reserves contribute to forage plants persistence at a greater extent than previously thought. Anyway, the role of C-reserves is not disparaged since carbohydrates support root respiration during a period of limited supply in reducing power and energy. No doubt that part of the future prospects will be to determine more precisely the specific implication of each organic N and C polymer constituent implied in re-growth recovery. As a consequence, informative results on putative differential responses between defoliation-tolerant and -intolerant species should be provided, and should be helpful to grassland managers for a new statement of agricultural practices.

REFERENCES

Avice, J.-C., A. Ourry, G. Lemaire and J. Boucaud. 1996a. Nitrogen and carbon flows estimated by 15N and 13C pulse chase labeling during regrowth of Medicago sativa L. Plant Physiol., in press.

Avice, J.-C., A. Ourry, J.J. Volenec, G. Lemaire and J. Boucaud. 1996b. Defoliation-induced changes in abundance and immuno-localization of vegetative storage proteins in taproot of Medicago sativa. Plant Physiol. Biochem., in press.

Bouchart, V., A. Ourry, J.-C. Simon and J. Boucaud. 1997. Seasonnal fluctuation of vegetative storage proteins and starch in Trifolium repens L. Proc. 18th Int. Grass. Cong., Winnipeg, Canada, in press.

Corre, N., V. Bouchart, A. Ourry and J. Boucaud. 1996. Mobilization of nitrogen reserves during regrowth of defoliated Trifolium repens L. and identification of potential vegetative storage proteins. J. Exp. Bot., in press.

Desjouis, M., F. Le Dily and J. Boucaud. 1996. Evidence for a polyamine-mediated control of soluble nitrogen mobilization during post-clipping re-growth of white clover (Trifolium repens L.). Plant Growth Reg., in press.

Gordon, A.J. and W. Kessler. 1990. Defoliation-induced stress in nodules of white clover. J. Exp. Bot. **41**: 1255-1262.

Graber, L.F., N.T. Nelson, W.T. Lueckel and W.B. Albert. 1927. Organic food reserves in relation to growth of alfalfa and other perennial herbaceous plants. in Research Bulletin 80, University of Wisconsin, Madison, WI.

Hendershot, K.L. and J.J. Volenec. 1993a. Taproot nitrogen accumulation and use in overwintering alfalfa. J. Plant Physiol. **141**: 68-74.

Hendershot, K.L. and J.J. Volenec. 1993b. Nitrogen pools in taproots of Medicago sativa L. after defoliation. J. Plant Physiol. **141**: 129-135.

Kaur-Sawhney, R., L.M. Shih, T. Cegielska and A.W. Galston. 1982. Inhibition of protease activity by polyamines. Relevance for control of leaf senescence. FEBS Lett. **145**: 345-349.

Ourry, A., J. Bigot and J. Boucaud 1989. Protein mobilization from stubble and roots, and proteolytic activities during post-clipping re-growth of perennial ryegrass. J Plant Physiol. **134**: 298-303.

Ourry, A., J. Boucaud and M. Duyme. 1990. Sink control of nitrogen assimilation during re-growth of ryegrass. Plant Cell Env. **13**: 185-189.

Staswick, P.E. 1988. Soybean vegetative storage protein structure and gene expression. Plant Physiol. **87**: 250-254.

Sung, H.I., L.F. Liu and C.H. Kao. 1994. The induction of a-amylase by sucrose starvation in suspension-cultured rice cells is regulated by polyamines. Physiol. Plant. **91**: 137-140.

Volenec, J.J., A. Ourry and B.C. Joern. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiol. Plant. **97**: 185-193.

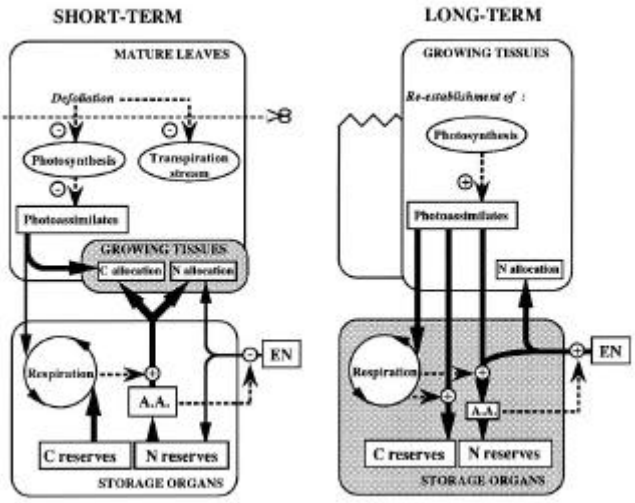

Figure 1
A simplified schematic view of short- and long-term physiological and biochemical responses of forage plants submitted to defoliation, exhibiting reserve mobilization and nutrient exchanges between source (white rectancles) and sink (grey rectangles) organs. The thin and bold arrows depict respectively decresed and increased fluxes of nutrients compared to undefoliated plants. Known controlled processes are pointed out with dotted arrows, supported by + (activation or increase) or - (down regulation or decrease). A.A., amino acids; EN, exogenous nitrogen.