Characterization of the sink/source transition in tobacco (Nicotiana tabacum L.) shoots in relation to nitrogen management and leaf senescence

Céline Mascaux, Marie-Hélène Valadier, Norbert Brugière, Jean-François Morot-Gaudry, Bertrand Hirel

Unité de Nutrition Azotée des Plantes, INRA, Centre de Versailles, Route de Saint Cyr, 78026 Versailles Cedex, France

Received: 11 October 1999 / Accepted: 20 January 2000

Abstract. The metabolic, biochemical and molecular events occurring during tobacco (Nicotiana tabacum) leaf ageing are presented, with a particular emphasis on nitrogen metabolism. An integrated model describing the source/sink relationship existing between leaves of different developmental stages along the main plant axis is proposed. The results of our study show that a tobacco plant can be divided into two main sections with regards to sink/source relationships. Sink-to-source transition occurs at a particular leaf stage in which a breakpoint corresponding to an accumulation of carbohydrates and a depletion of both organic and inorganic nitrogen is observed. The sink/source transition is also marked by the appearance of endoproteolytic activities and the induction of both cytosolic glutamine synthetase and NAD(H)-dependent glutamate dehydrogenase transcripts, proteins and activities. The role of the newly induced enzymes and the nature of the potential metabolic and developmental signals involved in the regulation of their expression during leaf senescence are discussed.

Key words: Glutamate dehydrogenase – Glutamine synthetase – Leaf senescence – Metabolic signals – Nicotiana (N metabolism) – Nitrogen metabolism

Introduction

Senescence is a phase of plant development which can be macroscopically observed in both annual and perennial plants by a progressive yellowing of the leaves, and is commonly defined as the sequence of biochemical and physiological events comprising the final stage of development until cell death (Smart 1994). Senescence is genetically controlled, e.g. it will occur at a given time in the life of the leaf, even if growth conditions are nearly optimal. Senescence may then be defined as the ordered degradation of cell constituents. The earliest and most drastic change in plant cellular structures during senescence is the breakdown of the chloroplasts which contain the photosynthetic machinery of the cell, carry out major biosynthesis and hold the majority of the leaf protein (Matile 1992). Consequently, a decrease in photosynthetic capacity during senescence is observed due to both Rubisco and chlorophyll degradation (Peterson and Huffaker 1975; Okada et al. 1992; Jiang et al. 1993; Matile et al. 1996; Ono and Watanabe 1997; Wingler et al. 1998). It is well known that the amount of Rubisco in a fully expanded leaf may account for at least 50% of total soluble protein and its degradation during leaf senescence supports the idea that this enzyme also represents an important cellular component in which nitrogen is stored during leaf expansion and which can be rapidly remobilized to sustain the growth of young developing tissues (Mae et al. 1983).

In the past few years an increasing number of studies have been undertaken in order to identify genes encoding enzymes or proteins that are specifically induced for the remobilization of nitrogen, carbon and minerals during leaf senescence (Feller and Fischer 1994; Smart 1994; Noodén et al. 1997). However, knowledge of the biochemical and molecular mechanisms controlling protein degradation and the subsequent process of nitrogen remobilization is still fragmentary since the different studies generally investigated a single enzymatic reaction in a given plant species (see Feller and Fischer 1994, for a review). As a result, only tentative models have been then proposed in order to assign a specific role to a particular enzyme in the whole-plant context (Buchanan-Wollaston 1997). Moreover, regulation of the biochemical changes occurring during the remobilization process is still poorly

Abbreviations: Fd-GOGAT = ferredoxin-dependent glutamate synthase; GABA = γ-amino-2-butyric acid; GDH = glutamate dehydrogenase; GS = glutamine synthetase; GS1 = cytosolic GS; GS2 = chloroplastic GS; ICDH = isocitrate dehydrogenase; NADH-GOGAT = NADH-dependent GOGAT; NR = nitrate reductase

Correspondence to: C. Mascaux;
E-mail: mascaux@versailles.inra.fr; Fax: +33-1-30833096
documented (Smart 1994; Gan and Amasino 1997; Noodên et al. 1997).

We therefore developed an integrated approach to study the various events related to nitrogen primary-assimilation or remobilization during the progression from young developing to old senescing leaves. Tobacco was chosen as a model plant system since leaf senescence is known to be independent of floral development (Thomas and Stoddart 1980). In addition, along the tobacco main axis, each leaf can easily be numbered and a single plant provides a large set of leaves in which there is a gradation of senescence. Such a model plant is then of particular interest in order to study the evolution of both nitrogen assimilation and remobilization with ageing, and to evaluate the potential involvement of the various metabolic pools as triggering signals.

We examined the main metabolic markers in leaves of increasing age by measuring the concentration of various nitrogen and carbon metabolites, the activity of the enzymes involved in nitrate reduction and ammonia assimilation, and the evolution of the amount of the corresponding proteins and transcripts.

The results obtained have allowed us to build up a comprehensive picture of the progressive transition occurring between nitrate assimilation and protein remobilization, both at the biochemical and molecular levels. This work proposes for the first time an integrated model describing the source/sink relationship existing between leaves at different developmental stages. In addition, it describes the progressive change in the various pools of metabolites, including carbohydrates, amino acids and inorganic nitrogen, in a whole-plant system. We have clearly shown that vegetative tobacco plants can be divided into two main sections with regards to the primary-assimilation and remobilization functions previously proposed in the literature. Interestingly, these sections are defined by a major break point in respect to both carbon and nitrogen pools. Both the putative role of these pools and their possible interaction is discussed in relation to the source/sink transition during leaf ageing.

Materials and methods

Plant material and growth. Tobacco (Nicotiana tabacum L. cv. Xanthi XHFD8; INRA, Versailles, France) was grown on a clay loam soil. From the base of the seedlings each emerging leaf was numbered and tagged. From a batch of 8-week-old plants, 8 plants of uniform development and numbering 10 leaves were selected. These were transferred to a controlled-environment growth chamber (16 h light, 350–400 nmol photons m⁻² s⁻¹, 26 °C; 8 h dark, 18 °C) and watered with N12 (10 mM NO₃⁻ and 2 mM NH₄⁺ solution (Cofc and Lesaint 1971). Plants were automatically watered for 1 min (flow rate for each plant: 50 ml min⁻¹) every 2 h. We selected 4 of the 11-week-old plants in the vegetative state of development, and harvested leaves numbered 9, 10, 11, 13, 15, 20, 25 and 30 (from bottom to top leaves) among the 35 leaves which had emerged. From each leaf, the main midribs were removed and 1-cm² sections of mesophyll tissue were randomly collected and pooled in two groups. One was weighed and then lyophilized to determine fresh and dry weights per area. The other was weighed, frozen and used to determine amounts of protein and chlorophyll per unit fresh weight and leaf area. The remaining mesophyll tissue was frozen in liquid nitrogen and immediately reduced to a homogenous powder which was stored at −80 °C and used for all further experiments. All the harvesting of fresh material was done concomitantly between 10 and 11 a.m. in order to estimate the highest level of extractable nitrate reductase (NR) activity.

Statistics. Results are presented as mean values (for three to four plants) with standard errors.

Chlorophyll and total protein determinations. Chlorophyll was estimated on crude leaf extracts according to Arnon (1949). Soluble protein was determined using a commercially available kit (Coomassie Protein assay reagent; Bio-Rad).

Metabolite extraction and analysis. Lyophilized plant material was used for metabolite extraction. Nitrate, NH₄⁺ and amino acids were extracted with 2% 5-sulfosalicylic acid (10 mg DW ml⁻¹; Ferrari-Méry et al. 1998). The total amino acid content and compositions of the individual amino acids were determined by ion-exchange chromatography (Rochat and Boutin 1989) on pooled samples extracted from an equal dry weight of material from each leaf range. The concentration of NO₃⁻ was determined according to the method of Cataldo et al. (1975). Free NH₄⁺ was determined by the phenol hypochlorite assay (Berthelot reaction). Sucrose, glucose, fructose, and starch were extracted with 1 M HClO₄ (1 ml per 5–10 mg DW of plant material) as described by Ferrari-Méry et al. (1998). The neutral sugars (glucose, fructose and sucrose) were measured enzymatically using a commercially available kit (Boehringer Mannheim). The starch content was determined as described by Ferrario-Méry et al. (1998).

Enzymatic assays. Enzymes were extracted from frozen leaf material stored at −80 °C. All extractions were performed at 4 °C. Nitrate reductase was extracted and the maximal extractable activity and activation state measured as described by Ferrario-Méry et al. (1998). The activity of NR was expressed as mmol NO₃⁻ h⁻¹ (mg protein)⁻¹. The activation state of NR is defined as the ratio of the activity measured in the presence of 10 mM MgCl₂ divided by the activity in the presence of 5 mM EDTA and was expressed as a percentage. Glutamine synthetase (GS) was measured according to the method of O’Neal and Joy (1973). The glutamate dehydrogenases NAD(H)-GDH and NADP(H)-GDH were measured as described by Turano et al. (1996) except that the extraction buffer was the same as for GS. The GDH activity was expressed as nmol NAD(H) used per min and mg protein. The activity of ferredoxin-dependent glutamate synthase (Fd-GOGAT) was measured as described by Suzuki-Méry et al. (1994) using methyl viologen as electron donor; Fd-GOGAT activity was expressed as nmol glutamate produced per min and mg protein. Endoproteolytic activities were measured according to Roy-Macaulay et al. (1992), and endoproteolytic activity was expressed as ΔA (mg protein)⁻¹ h⁻¹, ΔA being the increase in absorbance (A) of reaction supernatant at 330 nm.

Gel electrophoresis, gel staining procedure and protein blot analysis. Proteins were extracted from frozen leaf material in cold extraction buffer containing 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 0.5% (v/v) polyvinyl pyrrolidone (PVP), 0.1% β-mercaptoethanol (v/v) and 4 mM leupeptin. Protein concentrations were determined using the Bio-Rad protein assay. Proteins were separated on an SDS-PAGE acrylamide gel (Laemmli 1970). Equal amounts of protein (10 μg) were loaded in each track. The percentage of polyacrylamide in the running gels was 10% for GS and GDH polypeptide separation, and 7% for Fd-GOGAT and NADH-dependent GOGAT (NADH-GOGAT). Denatured proteins were electrophoretically transferred into nitrocellulose membranes. Polypeptide detection was performed using polyclonal antiserum raised against chloroplastic GS (GS2) of tobacco (Becker et al. 1992). Fd-GOGAT (Suzuki-Méry et al. 1994), NADH-GOGAT of maize (Hayakawa et al. 1994) and GDH of grape leaf (Loulakakis and Roubelakis-Angelakis 1990). Relative