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The AHAS gene of *Cichorium intybus* is expressed in fast growing and inflorescential organs

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Expression of acetohydroxy acid synthase (AHAS, EC 4.1.3.18) was analysed in biennial chicory (*Cichorium intybus* L. cv. Witloof) by northern blot analysis and enzyme assays. Young plantlets displayed high enzyme activity and AHAS mRNA accumulation in the root and the young leaves. Conversely, both enzyme activity and AHAS mRNA were undetectable in the mature tuberous taproot produced at the end of the first year. When such a taproot is grown in darkness, it develops an etiolated bud (the chicon) in which enzyme activity and AHAS mRNA are located in the neoformed lateral roots and the youngest central leaves. When this root is grown in the light, it produces a floral stem with many capitules. AHAS activities as well as AHAS mRNA were at the highest in capitules bearing developing seeds. However, AHAS seems strongly expressed in the young and developing tissues which need amino acids for protein synthesis and tightly associated to carbon influx whenever it is provided by photosynthesis or fructan remobilization.

Introduction

Acetohydroxyacid synthase also known as acetolactate synthase (AHAS, ALS, EC 4.1.3.18), catalyses the first step of the branched chain amino acid biosynthetic pathway. In the pathway leading to the synthesis of valine and leucine, it condenses two molecules of pyruvate yielding acetolactate, whereas in the pathway that yields isoleucine, it catalyses the condensation of one molecule of pyruvate and one molecule of α-ketobutyrate to produce 2-aceto-2-hydroxybutyrate (Umbarger 1978). Localized in the plastids of higher plants (Bekkaoui et al. 1993), the enzyme undergoes an allosteric inhibition by valine, leucine and at a lesser extent by isoleucine which are the end products of the biosynthetic pathway (Miflin and Cave 1972, Shield et al. 1996; for a review see Chipman et al. 1998). It is also inhibited by several classes of herbicides (Anderson and Hibberd 1985, Mazur and Falco 1989, Shimizu et al. 1994, Dewaele et al. 1997).

The biosynthesis of the branched chain amino acids primarily occurs in young tissues of different plant organs. This is sustained by the ubiquitous accumulation of AHAS mRNAs in growing organs (Singh and Matthews 1994). The number of AHAS genes varies according to the plant species. *Arabidopsis thaliana* contains one AHAS gene which is constitutively expressed, (Mazur et al. 1987). Regulation of the AHAS gene has been studied in species that possess several copies of the AHAS gene in their genome. The allotetraploid tobacco (*Nicotiana tabacum*) displays a simultaneous expression of the two genes *SuRA* and *SuRB* mainly in inflorescences and root apices (Keeler et al. 1993). In the allotetraploid cotton (*Gossypium hirsutum*), 4 of the 6 different AHAS genes identified, are organized in two very homologous pairs, one showing a low constitutive expression while the other one presents a highly specific expression restricted to anthers. The two last genes are less homologous and show a strong AHAS expression particularly in leaves, green pericarp, dry seeds and embryogenic callus tissue (Grula et al. 1995). The amphidiploid oilseed rape (*Brassica napus*) contains 5 genes (Rutledge et al. 1991). Two of the 5 AHAS copies are weakly but constitutively expressed (0.01–0.001% of total RNA), whereas an other one is only but strongly expressed in mature ovules and extraembryonary tissues. The other ones are truncated and result in a non-functional enzyme (Ouellet et al. 1992).

Abbreviations – AHAS, acetohydroxy acid synthase; BCAA, branched-chain amino acids; DAT, days after transfer; DOF, days of forcing; RH, relative humidity; TPP, thiamine pyrophosphate.

Chicory (*Cichorium intybus* L. var. Witloof, Asteraceae) displays a biennial cycle. During the first year, it produces a rosette of broad leaves and a tuberous taproot. Previous work showed that the development of the taproot is accompanied by changes in both the nitrogen and the carbohydrate metabolism. In young chicory, reduction of nitrate was observed mostly in the aerial part of the plantlets (Druart et al. 2000). As the root is thickening, proteins, fructans of the inulin type and free amino acids at a lesser extent accumulated, whereas nitrate assimilation is high in the young root (Dorchies and Rambour 1985, Limami et al. 1993, Van den Ende and Van Laere 1996). After a vernalization period, nitrogen and carbon stores are mobilized for the edification of a floral stem bearing bracts and inflorescences when exposed to a photoperiod of at least 16 h light/8 h darkness.

In commercial cultivation, the tuberous taproots are harvested between late summer and early autumn, stored at 0°C and subsequently grown at 18°C in darkness and 90% relative humidity (RH) in hydroponic systems. An etiolated bud forms within 3 weeks comprising a short axis bearing about 20 etiolated tightly overlapping leaves. It is named chicon or Belgian endive and is consumed as a salad. Previous work showed that during the forcing process, nitrate reduction occurs in the newly formed lateral roots but remains undetectable in either the tuberous root or in the chicon (Dorchies and Rambour 1983).

The branched-chain amino acids (BCAA) biosynthetic pathway has received a great deal of interest because several classes of herbicide inhibit AHAS, its key enzyme. The high potency of these herbicides has led to the production of herbicide resistant crops as well as a better understanding of the regulation of the BCAA biosynthesis in plants (Singh and Shaner 1995). Our work brings new insights on the expression of AHAS during the development of chicory, a biennial species, whose tuberous root is also grown in darkness to produce an etiolated bud. To perform this study, we used northern blot analysis and enzyme activity assays of AHAS.

Materials and methods

Chemicals

Unless specified otherwise, all chemicals used in the experimental process were purchased from Sigma Chemicals Co., St Louis, MO, USA.

Plant material

In vitro culture

Chicory seeds (*Cichorium intybus* L. var. Witloof cv. Flash) were surface-sterilized for 10 min in 0.1% $HgCl_2$ (w/v) and subsequently rinsed 3 times with sterile water. Aseptic seeds were then embedded in 0.2% (w/v) agar and germinated on solid growth medium containing Heller's salts (Heller 1953), 58 mM sucrose and 6 g l^{-1} agar. The growth chamber was maintained at 22 ± 1°C with a photoperiod 16/8 h (light/dark) and a light irradiance at the plant level of 30 µmol m^{-2} s^{-1} (Koninklijke Philips Electronics N.V., Amsterdam, The Netherlands, TLD 36W/84). After 3 days, the seedlings were transferred in individual tubes containing the above mentioned medium.

Greenhouse culture

Seeds were grown in pots containing compost. Six-month-old plants were vernalized for 6 weeks at 4°C and subsequently grown in a greenhouse at 20°C, a photoperiod 16/8 h (light/dark) and a light irradiance at the plant level of 250 µmol m^{-2} s^{-1} in order to initiate flowering.

Forcing process

Production of the etiolated bud named chicon is an agronomical deviation of the biennial chicory cycle. The tuberized taproots are harvested from fields, stored in a cold room at 0–5°C for at least 4 weeks and subsequently submitted to a dark hydroponic culture at 18°C and high RH for 21 days.

Genomic DNA extraction and DNA gel blot analysis

Genomic DNA was prepared according to Doyle and Doyle (1990) and subjected to restriction endonuclease digestion. Digested DNA was electrophoresed on 0.8% agarose gels and transferred to Hybond-N membranes (Amersham–Pharmacia Biotech UK Ltd., Buckinghamshire, UK) as described by Sambrook et al. (1989). Blots were hybridized at 42°C with a [^{32}P]-labelled AHAS probe of chicory (Dewaele et al. 1997). The probe was labelled by using the T7-Quick-prime® kit (Pharmacia) according to the manufacturer's specifications. The blots were washed twice at 55°C in 2 × SSC (0.3 M NaCl, 30 mM trisodium citrate), 0.1% SDS and subsequently exposed for 3 days to Kodak© X-Omat autoradiographic films.

Plant sampling

For each experiment, two independent sets of chicories were harvested, comprising 10–15 in vitro-grown plantlets. Two flowering plants were harvested and the organs collected on each plant were used as one set of samples. The same sampling procedure was performed on chicory roots harvested during the forcing process.

On each set of samples, two repetitions of AHAS assays and one RNA northern analysis were performed.

Extraction of RNA and northern blot analysis

Total RNA was extracted from material frozen in liquid nitrogen according to Thomas (1980). Denaturated RNA samples were electrophoresed on 1.2% agarose gels containing 3% formaldehyde and blotted on Hybond-N membranes (Amersham) using 20 × SSPE (3.6 M NaCl, 0.2 M Na_2HPO_4 pH 7.5, 0.2 mM EDTA pH 8). Hybridization was carried out at 60°C according to Church and Gilbert (1984) using the above mentioned probe. The membrane was rinsed two times with 20 mM Na_2HPO_4, 0.5% SDS at 60°C and subsequently exposed for 5 days to Kodak© X-Omat autoradiographic films.

Intensity of the bands of hybridization signal and BET-stained rRNAs was estimated after digitization using Scion Image Software beta-3b (available at the URL: http://www.scioncorp.com/). The results are expressed in arbitrary units with the highest intensity as reference.

AHAS assays

AHAS activity was extracted and assayed as described by Forlani et al. (1991) with slight modifications. One gram of plant material was powdered in liquid nitrogen and suspended in 5 ml (g fresh weight)$^{-1}$ of 50 m*M* potassium phosphate buffer pH 7.5 containing 0.5 m*M* dithiothreitol, 1 m*M* $MgCl_2$, 0.1 m*M* thiamine pyrophosphate (TPP), 0.01 m*M* FAD, 20% (v/v) glycerol, and 5% (w/v) insoluble polyvinyl polypyrrolidone. After a centrifugation for 20 min. (18 000 *g*, 4°C), the supernatant was filtered through one layer of Miracloth© (Calbiochem–Novabiochem Corporation, La Jolla, CA, USA) and used as the enzyme extract. Aliquots of this extract were incubated up to 90 min at 35°C in the presence of 10 m*M* potassium phosphate buffer pH 7.5, 20 m*M* sodium pyruvate, 0.5 m*M* $MgCl_2$, 50 μ*M* TPP and 5 μ*M* FAD. Sulphuric acid catalysed the decarboxylation of acetolactate to acetoin which was determined colorimetrically. Proper checks were performed to quantify the level of endogenous acetoin in the respective samples. Protein concentration was measured according to the Bio-Rad® Protein Assay protocol, using bovine serum albumin as standard.

AHAS specific activities were calculated as the mean of two independant repetitions of 5 determinations and expressed as pkat (mg protein)$^{-1}$.

Results

Determination of the AHAS gene copy number in *Cichorium* genome

To determine the number of AHAS gene copies, chicory genomic DNA was restricted with *Xho*I, which does not cut within the probe fragment and *Eco*RI, which cuts once within it. As depicted in Fig. 1, under high-stringency hybridization and wash conditions, only one hybridizing fragment was found using *Xho*I whereas two fragments were revealed using *Eco*RI . Total RNA hybridization with the same probe under high stringency conditions detected only a 2.2 kb transcript.

As *Cichorium intybus* is a true diploid (2n = 2x = 18), these results suggest that there is a single copy of AHAS gene per haploid genome, as in *Arabidopsis thaliana* (Haughn and Sommerville 1986).

AHAS regulation during the first year of the growth cycle

In vitro culture

In order to maintain controlled growth conditions, the first stages of development were carried out in vitro. The plantlets were harvested 10, 17, 24 and 31 days after they were transferred (DAT) in the individual tubes as described in Materials and methods. Plants were weighed and then subjected to AHAS enzyme activity assays (Fig. 2a) and mRNA accumulation was assayed by northern blot analysis (Fig. 2b).

Fresh weight was increasing during the time course of the experiment, which was stopped after day 31. Thereafter, the

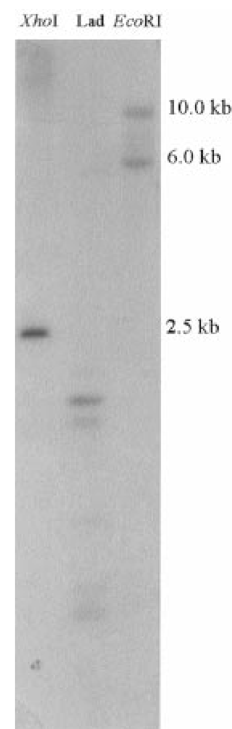

Fig. 1. Southern blot analysis of the AHAS gene. Ten micrograms of total DNA were digested by the indicated endonucleases. Molecular sizes are indicated on the side of the figure (Lad, ladder).

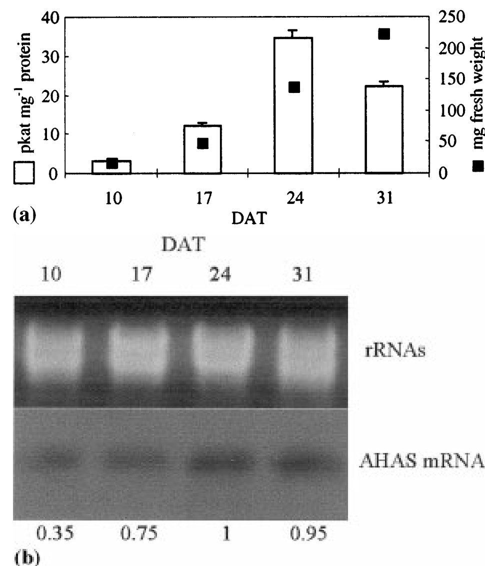

Fig. 2. AHAS activity (open bars) and average fresh weight (■) of in vitro-grown plantlets (a) and northern blot analysis of AHAS mRNA accumulation (b) during growth (DAT). Ethidium bromide-stained bands of rRNA are presented above the northern blot and numbers below represent an estimation of the hybridization signal with the most intense signal referred to as 1.

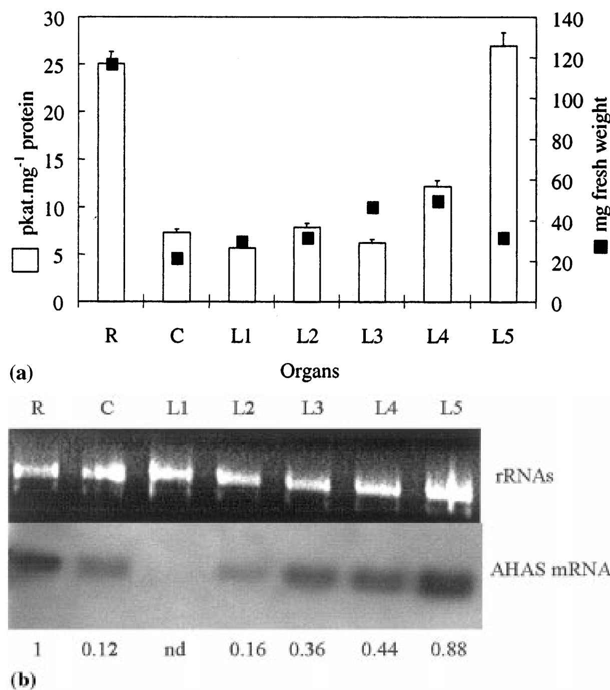

Fig. 3. AHAS activity (open bars) and average fresh weight (■) of the organs of 24-day-old plantlets (a) and northern blot analysis of AHAS mRNA accumulation (b). R, roots; C, cotyledons; L1, first leaf; L2, second leaf; L3, third leaf; L4, fourth leaf; L5, fifth leaf. Ethidium bromide-stained bands of rRNA are presented above the northern blot and numbers below represent an estimation of the hybridization signal as in Fig. 2 (nd, no signal detected).

plantlets showed necrosis on the young leaf probably because availability of nutriments became limiting. AHAS activities were rising until day 24 as AHAS mRNA content, but activity subsequently and strongly declined at 31 DAT, whereas mRNA content remained unchanged. Therefore, a tight relationship between the growth of the plantlets and the accumulation of AHAS mRNA occurred whereas the latter matched the AHAS activity excepted at the latest stage of the experiment.

Distribution of AHAS activity in the different organs of a 25-day-old plantlet

Twenty five DAT, in vitro-grown plantlets displaying the same developmental pattern (4 developed leaves plus a fifth emerging one) were selected. The different organs were dissected and subjected to AHAS assays. Their average fresh weight as well as their respective enzyme activity and their AHAS mRNA level are shown in Fig. 3a,b, respectively. Two types of organs displayed a high enzyme activity and mRNA accumulation: namely the roots and the young emerging leaf (L5). Whereas L5 represented one tenth of the total fresh weight of the plantlets, it exhibited the highest enzyme activity. When potential enzyme activity which is expressed as the activity of a particular organ taken as a whole was taken into account, the roots displayed half of the activity of the plantlet, whereas L5 leaves only represented 5% of that activity.

AHAS regulation during the second year of the chicory cycle

Organ repartition

After a vernalization period, the tuber produced a flowering stem bearing numerous inflorescences of capitules. The initiation of capitules is not synchronous, whereas flower initiation within one capitule is synchronous. Therefore some inflorescences were wilted and produced seeds, whereas other ones were still opening (Fig. 4). Thus, capitules can be harvested at different developmental stages from the same plant. So, AHAS was assayed on the tuberous taproot, the cauline leaves, the inflorescential bracts, stems, inflorescential buds, inflorescences with open flowers, and wilted inflorescences (Fig. 5a). mRNA accumulation was assayed by northern blot analysis (Fig. 5b). The different organs, which were assayed, fell in two categories. The first one comprising the tuberous root, the cauline leaves, the stem and the bracts (lanes 1–4) displayed both low AHAS and mRNA levels. The second category, which enclosed the inflorescences at the 3 developmental stages, displayed a high AHAS activity as well as high levels of mRNA (lanes 5–7). The organs of the first category are no longer in development. Conversely the organs of the second category are in constant evolution. Although the oldest inflorescences were wilted they were bearing developing seeds (Fig. 4b) which differentiated embryos and accumulated storage proteins.

AHAS activity during the formation of the chicon

During the forcing process, the cauline apical meristem of the taproot develops a short etiolated axis producing etiolated leaves, which remain tightly overlapped constituting the chicon. The chicon, the tuberous taproot and the lateral roots formed during forcing were harvested separately 1, 7 and 21 days after forcing was set on (DOF) (Fig. 6). AHAS activity and northern blot analysis were performed in the chicon, the lateral roots and the 3 parts (the top, the middle and the apical parts) of the taproot harvested at these 3 times (Fig. 7a,b). The tuberous taproot did not display any detectable AHAS activity or significant AHAS mRNA accumulation in any part, which was assayed. Conversely, the chicon as well as the lateral roots displayed high enzyme activity and AHAS mRNA accumulation.

AHAS activity in the leaves of the chicon

At 21 DOF, the chicon is fit for marketing. At this stage, it comprises more than 20 leaves which were sampled and categorised into 5 experimental sets comprising leaves 1–5, 6–10, 11–15, 16–20 and finally the central young leaves surrounding the apex respectively. Therefore, the first set consisted of the 5 outer oldest leaves whereas the successive sets consisted of further formed leaves. The highest AHAS activity was detected in the fifth set comprising the small internal leaves, which corresponded to less than 5% of the fresh weight of the chicon (Fig. 8a). Similarly, northern blot analysis showed that AHAS mRNAs were accumulating at a high level in these leaves (Fig. 8b). Therefore AHAS activities and mRNA accumulation displayed a decreasing

gradient extending from the young internal leaves to the outer oldest ones.

Discussion

Restriction analysis of the genomic DNA of chicory digested with either *Xho*I or *Eco*RI favoured the view of the presence of a unique AHAS gene per haploid genome in chicory. As hybridization was performed under high stringency, the existence of other genes cannot be definitely ruled out. However, northern blot analysis also revealed a unique detectable signal. Moreover, in heterozygous sulfonylurea resistant-sensitive chicory, both AHAS alleles were shown to be expressed (Dewaele et al. 1997). AHAS multigenic families seem to be positively correlated to the degree of ploidie of the genomes as was shown in tobacco, cotton or oilseed rape (Ouellet et al. 1992, Keeler et al. 1993, Grula et al. 1995).

In chicory, as in other species (Singh and Matthews 1994), expression of AHAS was positively correlated with fast growing organs. Thus, the young leaves and the roots of plantlets grown in vitro, as well as the growing inflorescences of the flowering chicory displayed a high enzyme activity as well as a high AHAS mRNA content. However, as the growth was slowed down in plants grown in vitro because the availability of nutrients might be limiting, the enzyme activity declined whereas the level of AHAS mRNA remained stable. Besides, photosynthesis might be unable to provide the biosynthetic pathways with sufficient carbon. Indeed, increasing the sucrose concentration from 58 up to 87 m*M* in the growth medium as an offset to the low photosynthetic activity, resulted in a decreased AHAS activity, whereas both growth and RNA accumulation were unaffected (data not shown). Thus, some post-transcriptional or post-translational mechanisms could prevail over transcriptional control. A mechanism that could be involved in low AHAS activity whereas AHAS mRNA level remained high is the lack of a possible small regulatory subunit (Singh and Shaner 1995, Chipman et al. 1998). Even if the existence of the AHAS small subunit in plant has been demonstrated (Hershey et al. 1999), presently little is known about how it may regulate the activity of the enzyme.

The organs of the young chicory that displayed the highest activity and AHAS mRNA content, were the roots and the youngest leaf. Both roots and young leaves are known to have an important sink strength toward carbohydrate produced in the old leaves. Therefore, AHAS activity seems to be tightly correlated to the carbon flow. The need is toward the BCAA biosynthesis as the other amino acids are implicated in the sink strength of the root and the young leaf. Young tissues of the root and the youngest leaf are not autotrophous toward carbohydrate and mostly depend on photosynthate influx provided by older leaves (Turgeon 1989).

In young plant organs, the dividing and the undifferentiated cells are in need of making structural proteins, enzymes

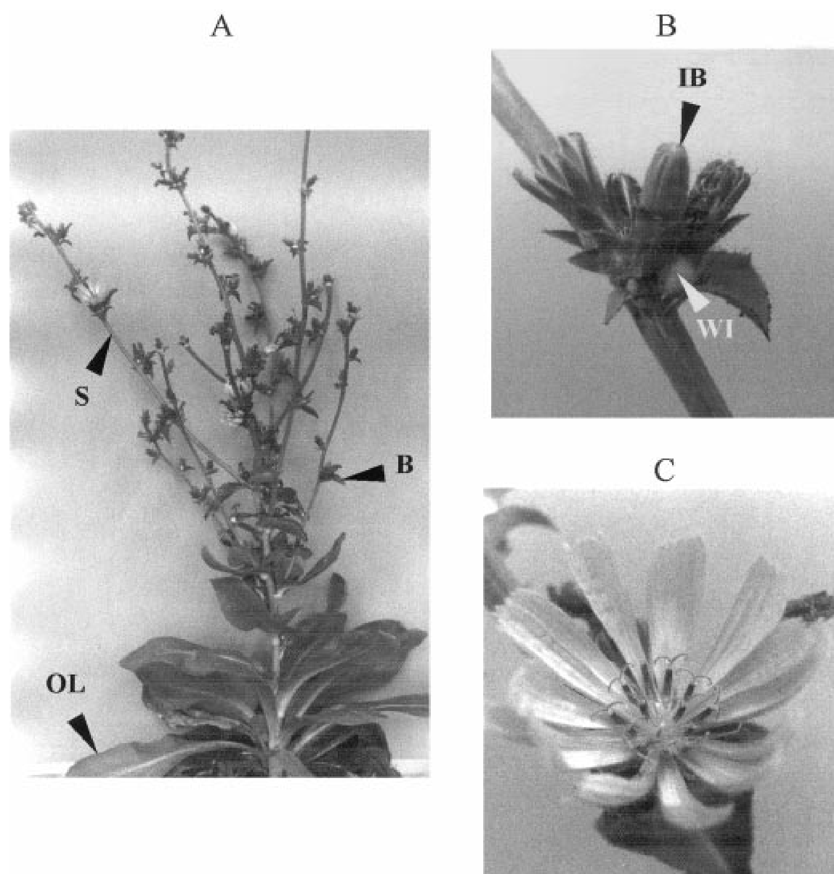

Fig. 4. Flowering chicory. (A) Global view of the plant: OL, old leaf; B, bract; S, stem. (B) A group of capitules: IB, inflorescential bud; WI, wilted inflorescence. (C) An open inflorescence.

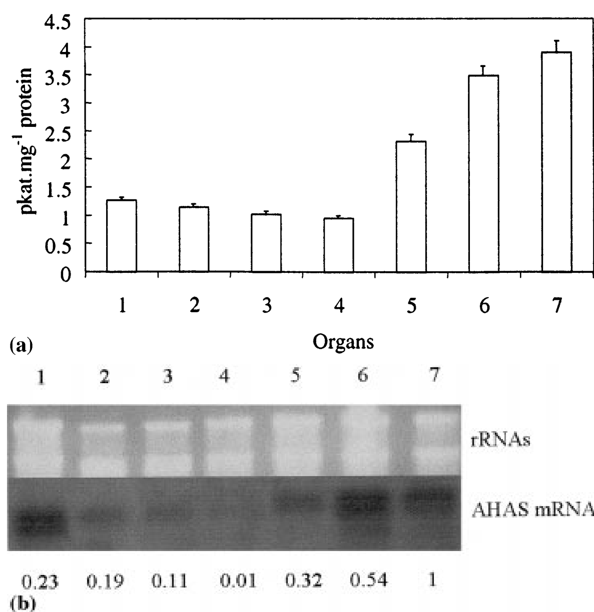

Fig. 5. AHAS activity (a) and northern blot analysis of AHAS mRNA accumulation (b) measured in the organs of flowering chicory. Lane 1, tuberous root; lane 2, old leaves; lane 3, stem; lane 4, bracts; lane 5, inflorescential bud; lane 6, open inflorescences; lane 7, wilted inflorescences. Ethidium bromide-stained bands of rRNA are presented above the northern blot and numbers below represent an estimation of the hybridization signal as in Fig. 2.

as well as proteins involved in the control of gene expression. In the oldest organs which comprise a high set of differentiated cells, AHAS activity might be maintained in a minimal state sufficient to support the protein turnover. Moreover, oldest organs can provide a source of amino acids and ammonia via the action of proteases (Callis 1995) and biodegradating enzymes such as degradating forms of threonine dehydratase which catalyse the dissociation of threonine and serine to produce ammonia and 2-ketobutyrate (Singh 1999).

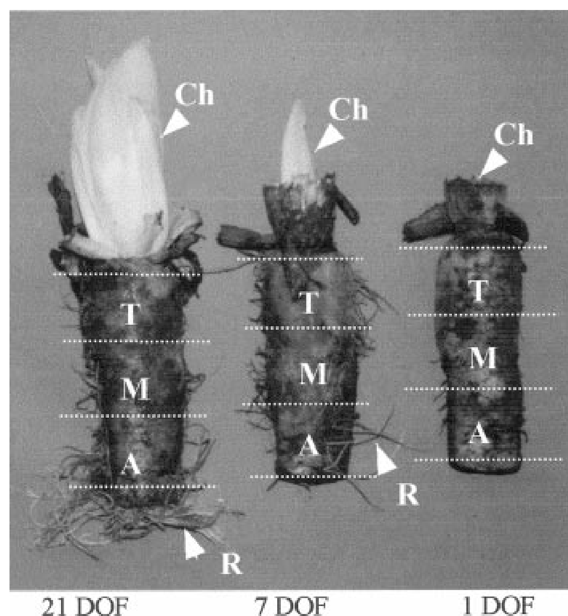

Fig. 6. Tuberous roots during the forcing process (DOF). Ch, chicon; T, top of the root; M, middle of the root; A, apex of the root; R, rootlets.

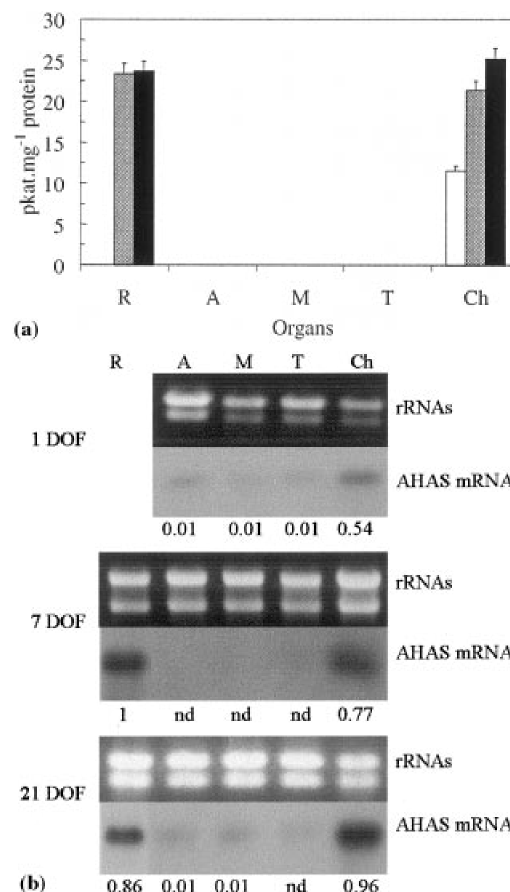

Fig. 7. AHAS activity (a) and northern blot analysis of AHAS mRNA accumulation (b) measured during the forcing process of the tuberous root (Ch, chicon; T, top of the root; M, middle of the root; A, apex of the root; R, rootlets; open bars, 1 DOF; grey bars, 7 DOF; black bars, 21 DOF). Ethidium bromide-stained bands of rRNA are presented above the northern blot and numbers below represent an estimation of the hybridization signal as in Fig. 2 (nd, no signal detected).

In light-grown chicory committed to flower, the vegetative organs displayed low enzyme activities and mRNA content, whereas high activities and mRNA content occurred in the capitules bearing opening flowers or immature seeds which accumulated proteins. This fits with the data of Keeler et al. (1993) who observed a high transcription of the AHAS gene in mature flowers, and Muhitch (1988) who observed a strong AHAS activity after pollination and during the synthesis of storage proteins in endosperm and embryos of maize. Besides, the remaining question is at what extent does hydrolysis of fructans stored in the taproot provide the young developing organs with carbon skeletons compared to the photosynthates.

In dark-grown plants, both the enzyme activity and AHAS mRNA accumulation occurred in lateral roots or in the young leaves formed during the forcing process, indicating again a positive relation between active growth and

AHAS expression. The tuberous root which is forced, contains fructans and N compounds which correspond to about 80–85% and 1%, respectively, of the dry weight (Limami et al. 1996). N is stored as vegetative storage proteins and amino acids, arginine being predominant. Amino acids were shown to be mobilized at the onset of the forcing process. The N content of the new produced leaves of the chicon is quite totally composed of tuberous root remobilized N, with a slight proportion of exogenous N (Fouldrin and Limami 1993, Limami et al. 1993). As AHAS catalyses the condensation of two molecules of pyruvate, fructan breakdown probably constitutes the source of pyruvate needed for BCAA biosynthesis and besides, the source of carbon skeletons for the cauline apex, the leaf primordia and the leaves growth. So, the remobilization of stored nitrogen and the fructan breakdown may intensively contribute to the biosynthesis of the BCAA during the forcing process, in the newly formed leaves and rootlets.

In conclusion, the AHAS gene is highly transcribed in these tissues grown in darkness, indicating that light did not directly control its expression, as it does on *cab* or *RbCs* genes whose products encoded by nuclear genes are also targeted to the chloroplasts (Batschauer 1998). So, it appears that, whatever source of carbon is provided, the AHAS expression is located in actively dividing cells of the youngest organs.

In dark-grown chicories, pyruvate, one of the substrates of AHAS, is only provided by the hydrolysis of fructans stored in the tuberous root. Nevertheless, in light-grown chicories committed to flower, the proper contribution of photosynthesis and remobilization of fructans to the AHAS activity remains unclear.

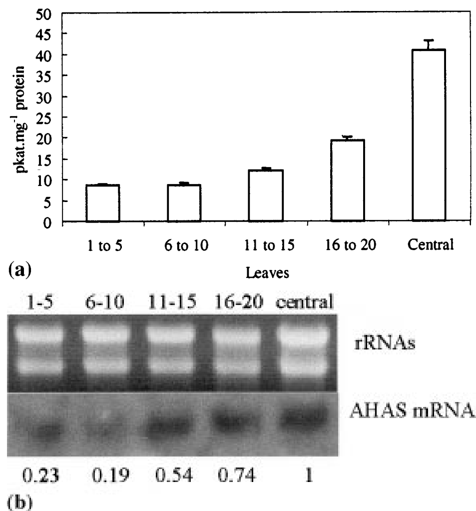

Fig. 8. AHAS activity (a) and northern blot analysis of AHAS mRNA accumulation (b) measured in the leaves of a 21-day-old chicon. Ethidium bromide-stained bands of rRNA are presented above the northern blot and numbers below represent an estimation of the hybridization signal as in Fig. 2.

References

Anderson PC, Hibberd KA (1985) Evidence for interaction of an imidazolinone herbicide with leucine, valine, and isoleucine metabolism. Weed Sci 33: 479–483

Batschauer A (1998) Photoreceptors of higher plants. Planta 206: 479–492

Bekkaoui F, Schorr P, Crosby WB (1993) Acetolactate synthase from *Brassica napus*: Immunological characterization and quaternary structure of the native enzyme. Physiol Plant 88: 475–484

Callis J (1995) Regulation of protein degradation. Plant Cell 7: 845–857

Chipman D, Barak Z, Schloss JV (1998) Biosynthesis of 2-aceto-2-hydroxy acids: Acetolactate synthases and acetohydroxyacid synthases. Biochim Biophys Acta 1385: 401–419

Church G, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81: 1991–1995

Dewaele E, Forlani G, Degrande D, Nielsen E, Rambour S (1997) Biochemical characterisation of chlorsulfuron resistance in *Cichorium intybus* L. var. Witloof. J Plant Physiol 151: 109–114

Dorchies V, Rambour S (1983) Activité de la nitrate réductase mesurée in vivo chez *Cichorium intybus* (var. Witloof) au cours du forçage. Physiol Vég 21: 705–713

Dorchies V, Rambour S (1985) Activité nitrate réductase chez *Cichorium intybus* (var. Witloof) à différents stades de développement et dans les tissus cultivés in vitro. Physiol Vég 23: 25–35

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15

Druart N, Goupil P, Dewaele E, Boutin JP, Rambour S (2000) Nitrate assimilation in chicory roots which acquire radial growth. J Exp Bot 51: 539–544

Forlani G, Nielsen E, Landi P, Tuberosa R (1991) Chlorsulfuron tolerance and acetolactate synthase activity in corn (*Zea mays* L.) inbred lines. Weed Sci 39: 553–557

Fouldrin K, Limami A (1993) The influence of ($^{15}NO_3$) supply to chicory (*Cichorium intybus* L.) plants during forcing on the uptake and remobilization of N reserves for chicon growth. J Exp Bot 44: 1313–1319

Grula JW, Hudspeth PL, Hobbs SL, Anderson DM (1995) Organisation, inheritance and expression of acetohydoxy acid synthase genes in the cotton allotetraploid *Gossypium hirsutum*. Plant Mol Biol 28: 837–846

Haughn GW, Sommerville CR (1986) Sulfonylurea-resistant mutant of *Arabidopsis thaliana*. Mol Gen Genet 204: 430–434

Heller R (1953) Recherche sur la nutrition minérale de tissus végétaux cultivés in vitro. Ann Sci Nat Biol Vég 14: 1–223

Hershey HP, Schwartz LJ, Gale JP, Abell LM (1999) Cloning and functional expression of the small subunit of acetolactate synthase from *Nicotiana plumbaginifolia*. Plant Mol Biol 40: 795–806

Keeler SJ, Sanders P, Smith JK, Mazur BJ (1993) Regulation of tobacco acetolactate synthase genes expression. Plant Physiol 102: 1009–1018

Limami A, Roux L, Laville J, Roux Y (1993) Dynamic of nitrogen compounds in the chicory (*Cichorium intybus*) tuberised taproot during the growing season and cold storage period. J Plant Physiol 141: 263–268

Limami A, Dufossé C, Richard-Molard C, Fouldrin K, Roux L, Morot-Gaudry JF (1996) Effect of exogenous nitrogen ($^{15}NO_3$) on utilization of vegetative storage proteins (VSP) during regrowth in chicory (*Cichorium intybus*). J Plant Physiol 149: 564–572

Mazur BJ, Falco SC (1989) The development of herbicide-resistant crops. Annu Rev Plant Physiol 40: 441–470

Mazur BJ, Chui CF, Smith JK (1987) Isolation and characterisation of plant genes coding for acetolactate synthase, the target enzyme of two classes of herbicide. Plant Physiol 85: 1110–1117

Miflin BJ, Cave PR (1972) The control of leucine, isoleucine and valine biosynthesis in a range of higher plants. J Exp Bot 23: 511–516

Muhitch MJ (1988) Acetolactate synthase activity in developing maize (*Zea mays* L.) kernels. Plant Physiol 86: 23–27

Ouellet T, Rutledge RG, Miki BL (1992) Members of the acetohydroxy acid synthase multigene family of *Brassica napus* have divergent patterns of expression. Plant J 2: 321–330

Rutledge R, Ouellet T, Hattori J, Miki B (1991) Molecular characterization and genetic origin of the *Brassica napus* acetohydroxyacid synthase multigen family. Mol Gen Genet 229: 31–40

Sambrook J, Fritch EF, Maniatis T (1989) Molecular Cloning, A Laboratory Manual, Vol. 2. Cold Spring Harbor Laboratory, New York, NY, pp 9.45–9.46. ISBN 0-87969-309-6

Shield DL, Bengston AS, Brockman JP, Butler JH, Kraatz GW, Lamoreaux RJ, Subramanian MV (1996) Patterns of cross-tolerance to herbicides inhibiting acetohydroxyacid synthase in commercial corn hybrids designed for tolerance to imidazolinones. Crop Sci 36: 274–278

Shimizu T, Nakayama I, Wada N, Nakao T, Abe N (1994) Kinetic studies on the inhibition of acetolactate synthase by pyrimidinylsalicylic acids. J Pestic Sci 19: 257–266

Singh BK (1999) Biosynthesis of valine, leucine, and isoleucine. In: Singh BK (ed) Plant Amino Acids. Marcel Dekker Publisher, New York, NY, pp 227–247. ISBN 0-8247-0204-2

Singh BK, Matthews BF (1994) Molecular regulation of amino acid biosynthesis in plants. Amino Acids 7: 165–174

Singh BK, Shaner DL (1995) Biosynthesis of branched chain amino acids: from test tube to field. Plant Cell 7: 935–944

Thomas PS (1980) Hybridization of denaturated RNA and DNA fragments transferred on nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5205

Turgeon R (1989) The sink-source transition in leaves. Annu Rev Plant Physiol 40: 119–138

Umbarger HE (1978) Amino acid biosynthesis and its regulation. Annu Rev Biochem 47: 533–606

Van den Ende W, Van Laere A (1996) Fructan synthetizing and degradating activities in chicory roots (*Cichorium intybus* L.) during field-growth, storage and forcing. J Plant Physiol 149: 43–50

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