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ORIGINAL ARTICLE

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An isoleucine-leucine substitution in chloroplastic acetyl-CoA carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim

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Abstract The cDNAs encoding chloroplastic acetyl-CoA carboxylase (ACCase, EC 6.4.1.2) from three lines of *Setaria viridis* (L. Beauv.) resistant or sensitive to sethoxydim, and from one sethoxydim-sensitive line of *Setaria italica* (L. Beauv.) were cloned and sequenced. Sequence comparison revealed that a single isoleucine-leucine substitution discriminated ACCases from sensitive and resistant lines. Using near-isogenic lines of *S. italica* derived from interspecific hybridisation, we demonstrated that the transfer of the *S. viridis* mutant ACCase allele into a sethoxydim-sensitive *S. italica* line conferred resistance to this herbicide. We confirmed this result using allele-specific polymerase chain reaction and showed that a single copy of the mutant allele is sufficient to confer resistance to sethoxydim. We conclude that a mutant allele of chloroplastic ACCase encoding a leucine residue instead of an isoleucine residue at position 1780 is a major gene of resistance to sethoxydim.

Keywords Acetyl-CoA carboxylase (fatty acid biosynthesis) · Herbicide · Mutation (acetyl-CoA carboxylase) · Resistance (herbicide) · *Setaria* (herbicide resistance)

Abbreviations ACCase: acetyl-CoA carboxylase · APP: aryloxyphenoxypropionate · CHD: cyclohexanedione · CT: carboxyl transferase · PCR: polymerase chain reaction

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Introduction

Acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) is a key enzyme in fatty acid biosynthesis in eukaryotes and prokaryotes (Harwood 1988). In plants, two forms of ACCase have been identified that are located in the chloroplast, the primary site of plant fatty acid biosynthesis, and in the cytosol (Sasaki et al. 1995; Konishi et al. 1996), respectively. In most plants, chloroplastic ACCase is a "prokaryotic-type", multi-subunit enzyme. Subunits of the prokaryotic ACCase are encoded in the nuclear DNA, except the β-subunit of carboxyl-transferase (CT), which is encoded by a chloroplastic gene (Konishi et al. 1996). In the Gramineae (Konishi et al. 1996) and the Geraniaceae (Christopher and Holtum 2000), chloroplastic ACCase is a "eukaryotic-type", multifunctional enzyme of around 250 kDa. The cytosolic ACCase in all plants studied so far is of the eukaryotic type. Genes for eukaryotic-type ACCases are nuclear. In the Gramineae, two distinct genes encode cytosolic and chloroplastic ACCases. This has been demonstrated in wheat (Gornicki et al. 1994, 1997; Podkowinski et al. 1996), and suggested for maize (Egli et al. 1993) and for the diploid gramineous weeds *Lolium multiflorum* Lam. (Evenson et al. 1997) and *Alopecurus myosuroides* Huds. (Menéndez and De Prado 1999), as a result of identification of two eukaryotic-type ACCase isoenzymes.

Chloroplastic ACCase is a vital point of plant metabolism (Ohlrogge and Jaworski 1997). Two chemically dissimilar classes of selective graminicide herbicides: aryloxyphenoxypropionates (APPs) and cyclohexanediones (CHDs) block fatty acid biosynthesis in the Gramineae by inhibition of their chloroplastic ACCase, causing plant death (Burton et al. 1989). Prokaryotic-type ACCase and cytosolic, eukaryotic-type ACCase are insensitive and significantly less sensitive, respectively, to CHDs and APPs than chloroplastic, eukaryotic-type ACCase (Egli et al. 1993; Alban et al. 1994). Thus, plants other than members of the Gramineae (except

those in the Geraniaceae) are insensitive to these herbicides, as are most other eukaryotes and prokaryotes. Because of the frequent use of APPs and CHDs, spontaneous resistance has appeared in 25 gramineous weed species (Heap 2001). Alteration of the chloroplastic, eukaryotic-type ACCase is the most frequent cause of resistance (e.g. Evenson et al. 1997; Shukla et al. 1997; Menéndez and De Prado 1999; see also Devine and Shukla 2000, for a review). This has been extensively documented based on biochemical evidence (Devine and Shukla 2000; see also Heap 2001, for a compilation of references). A recent report indicated that a 400-amino-acid region, encompassing the CT domain of wheat chloroplastic ACCase, is involved in insensitivity to both CHD and APP herbicides (Nikolskaya et al. 1999). However, to date, no mutation responsible for resistance to some of these herbicides has been identified.

In this paper, we report the molecular basis of green foxtail (*Setaria viridis* L. Beauv.) resistance to the CHD herbicide sethoxydim caused by insensitivity of the target enzyme. *Setaria viridis*, a wild relative of the cultivated foxtail millet (*S. italica* L. Beauv.), is a major weed of wheat, maize and soybean. Biotypes of *S. viridis* have developed resistance against a range of ACCase-inhibiting herbicides (Heap and Morrison 1996). In particular, a biotype of *S. viridis* displaying a very high level of resistance to sethoxydim due to altered chloroplastic ACCase has been reported (Heap and Morrison 1996; Shukla et al. 1997).

Materials and methods

Plant material

We used interspecific hybridisation to transfer a mutated gene encoding chloroplastic ACCase from Canadian green foxtail (*Setaria viridis* L. Beauv.), line UM131, that is highly resistant to sethoxydim (Heap and Morrison 1996; Shukla et al. 1997), into cultivated foxtail millet (*S. italica* L. Beauv.). Seeds from *S. viridis* line UM131 were provided by Dr. I.N. Morrison (University of Manitoba, Winnipeg, Canada). Interspecific crossings were performed as described by Wang and Darmency (1997), using the male-sterile, sethoxydim-sensitive Shda1 line of *S. italica* as the female parent. Resistant F1 hybrid lines were backcrossed seven times with the sensitive *S. italica* parental line Shda1 to obtain BC7, as described by Wang and Darmency (1997). At this stage, the hybrid population progeny had on average only 0.39% of the genome of the UM131 *S. viridis* parental line, and were considered isogenic. Hybrid lines D97-6a and D97-5a, sensitive and resistant to sethoxydim, respectively, were selected after three self-pollinating generations using herbicide selection (Wang and Darmency 1997).

Herbicide sensitivity assay

A rapid seed-bioassay was used to assess sethoxydim sensitivity of *Setaria* sp. seedlings. Seeds (100) were deposited on filter paper placed on 0.7-cm-diameter glass tubes in 13.5-cm-diameter glass Petri dishes containing 60 ml sethoxydim solution (33 μM). Sethoxydim solutions were prepared from the commercial herbicide Fervinal (Schering-Plough, Levallois-Perret, France; 192 g l^{-1} sethoxydim) using deionised water. Plates were incubated for 72 h at 27 °C in darkness. A seedling was considered sensitive if the length of its coleoptile was ≤ 2 cm, and resistant if it was >2 cm. To obtain plant material for ACCase cloning experiments, resistant or sensitive seedlings were collected from sethoxydim plates, rinsed with deionised water and cultivated in a greenhouse as described by Wang and Darmency (1997) until they had three or four fully expanded leaves.

Extraction of RNA and cDNA synthesis

Total RNA from *S. italica* line Shda1 was extracted from one single plant with three expanded leaves using the RNeasy extraction kit (Qiagen) and following the manufacturer's instructions. The final elution step was performed in 100 μl RNase-free water. Single-stranded cDNA was prepared from 13 μl RNA solution using the Omniscript reverse-transcription kit (Qiagen) and either the poly-dT primer NotIdT or a gene specific primer (Table 1). The

Table 1 Primers

Primer	Sequence (5′ to 3′)	Location[a]	Experiment
NotIdT	AACTGGAAGAATTCGCGGCCGCAGGAATTTTTTTTTTTTTTTTT	NA[b]	Reverse-transcription
ACS1R	AAGGCTTCTTGCGATGCTTGCACGGAGTTC	3333–3304	”
ACS3R	TTTGCATAGTTGTTATTGTTTGTTCCACC	608–580	”
ACSRT1	TAACTGTCTGTCACTCAGCTCCCCCAAGC	7144–7116	”
ACSRT2	GGAGATATGTATGTCTCGATCACTTTCTGC	3488–3459	”
ACA1	GGTTGTAGACATTGTTTTGTCTCACCAGGG	3108–3137	Degenerate PCR
ACV1R	AGGTAAATGTACTGAAAICCACGTTCAGG	5210–5182	”
ACC1	ATGGCIACICCIGARGAYATGMG	508–530	”
ACS2R	AGAAAAGCGAACCAACAGATCCCTGTAAGC	3231–3199	Gene-specific PCR
ACS1	GGTGCTAGGATTGGCATAGCCGATGAAGTG	5113–5142	”
ACS4R	CAGCAATTCTAATGTGTTCTGCATTTATCC	559–530	”
ACSP1	GTGAACTCCGTGCAAGCATCGCAAGAAGCC	3302–3331	”
ACSP1R	CAGATATGATCAATATCCTTCTATGTATG	7048–7020	”
ACSP2	GGCACTTTGCCTTTGAGAAAATATTTAATTGTG	–54 to –22	”
ACSP2R	AAATAAGTGCATCTTCGACAGGTAATGGG	3427–3399	”
ACSA	GGGCTCAATGACATTGGCATGGTAGC	4909–4934	”
ACSAR	ACCACACTTGTCCAGCCCGAGGAAC	5995–5971	”
SETS1	TGGGCTTGGTGTTGAGAATA	5319–5338	Allele-specific PCR
SETR1R	AGCAGCACTTCCATGTAG	5359–5338	”

[a]The first nucleotide in the *S. italica* ACCase coding sequence is number 1
[b]Not applicable

manufacturer's instructions were followed, except that samples were incubated at 37 °C for 2 h instead of 60 min.

Cloning a fragment of the *S. italica* chloroplastic ACCase gene

All primers used in this work are shown in Table 1. All PCR amplifications were performed in 20 µl using the proofreading DyNAzyme EXT (Finnzymes, Espoo, Finland) in its provided buffer, with a final concentration of 250 µM of each dNTP. Primers ACA1 and ACV1R targeted highly conserved regions of eukaryotic-type, chloroplastic ACCase coding sequences from wheat (Gornicki et al. 1997; GenBank Acc. No. AF029895), maize (Egli et al. 1995; GenBank Acc. No. U19183) and oat (GenBank Acc. No. AF072737). They were used at a final concentration of 0.2 µM each. The cycling program comprised 37 cycles each consisting of 30 s at 95 °C, 45 s at 60 °C and 2 min at 72 °C, followed by a final step of 10 min at 72 °C. A single amplicon of about 2,100 bp was obtained from the NotIdT reverse-transcription mix and cloned into the vector pGEM-T (Promega). To exclude PCR errors, three different clones were sequenced on both strands using extension of specific primers. Degenerate primer ACC1, which correspond to the amino acid sequence MATPEDMR, targeted a conserved region of the biotin-carboxylase domain of plant eukaryotic-type ACCases. Primers ACS1R and ACS2R were designed based on the 5′-end sequence of the fragment obtained using primers ACA1 and ACV1R. Primers ACC1 and ACS2R were used at a final concentration of 0.75 µM and 0.1 µM, respectively. The cycling program was as before, except that annealing and extension times were 30 s and 3 min, respectively. A predominant amplicon of about 2,700 bp was obtained from the ACS1R reverse-transcription mix and purified from a 0.6% (w/v) agarose gel run at 65 V in 1× Tris-acetate EDTA buffer using the Concert gel extraction system (Gibco BRL). Cloning and sequencing were as before.

3′- and 5′-Rapid amplification of cDNA ends (RACE)

The 3′ end of the *S. italica* chloroplastic ACCase gene was obtained using primer NotIdT and primer ACS1, designed based on the 3′-end sequence of the fragment obtained using primers ACA1 and ACV1R, at a final concentration of 0.5 µM and 0.2 µM, respectively. The cycling program was as for primer pair ACC1/ACS2R. A single amplicon of about 2,600 bp was obtained from the NotIdT reverse-transcription mix. Cloning and sequencing were as before.

Primers ACS3R and ACS4R were designed based on the 5′ end of the fragment obtained using primers ACC1 and ACS1R. A 5′-RACE system kit (Gibco-BRL) was used according to the manufacturer's protocol. Starting RNA material consisted of 13 µl of Shda1 RNA solution. First-strand cDNA was prepared using primer ACS3R, followed by the addition of a homopolymeric dC tail. Primer AAP provided in the kit was used for PCR together with primer ACS4R, at a final concentration of 0.2 µM each. The cycling program was made up of one denaturation step of 30 s at 95 °C, followed by 37 cycles of 30 s at 95 °C, 30 s at 59 °C and 1 min at 72 °C, and terminated by a final step of 10 min at 72 °C. A unique amplicon of about 900 bp was obtained, which was cloned and sequenced as before.

Sequence comparison of cDNAs encoding chloroplastic ACCase

Since *S. viridis* and *S. italica* are closely related species, two sethoxydim-sensitive Canadian lines of *S. viridis* collected in 1990 (line D90-19: Deloraine, Manitoba, Canada, and line D90-58: Oak River, Manitoba, Canada, both provided by Dr. I.N. Morrison) were included in this study in order to discriminate between nucleotide polymorphism related to resistance and interspecific nucleotide polymorphism. Total RNA was extracted as before from a plant with three (*S. italica* introgression lines D97-5a and D97-6a) or four (*S. viridis* lines UM131, D90-19 and D90-58) expanded leaves. From each RNA sample, two overlapping cDNA fragments of about 3,500 bp each were generated. One was obtained from ACSRT1 reverse-transcription mixes using primers ACSP1 and ACSP1R, and the second from ACSRT2 reverse-transcription mixes using primers ACSP2 and ACSP2R. All primers were used at a final concentration of 0.2 µM each. The cycling program used for both primer pairs consisted of 37 cycles of 30 s at 95 °C, 30 s at 58 °C and 4 min at 72 °C, followed by a final step of 10 min at 72 °C. A single predominant amplicon of about 3,500 bp was obtained in all cases. Extraction from gels, cloning and sequencing were as before.

Nucleotide sequence assembling and translation were performed using the Seqaid II software (D.D. Rhoads and D.J. Roufa, Kansas State University, Manhattan, Kan., USA). Aligning was done using the Multalin software (Corpet 1988).

Allele-specific PCR

DNA was extracted from resistant and sensitive seedlings obtained as described under *Herbicide sensitivity assay*. A 1-cm section of the coleoptile of each seedling was cut and placed in a microcentrifuge tube containing 150 µl of the extraction buffer described by Saini et al. (1999). The coleoptile sections were crushed using disposable micropipette tips. Tubes were closed and placed in a water bath at 95 °C for 6 min, transferred into ice for 5 min and vortexed for 15 s. DNA extracts were kept at –20 °C prior to PCR analysis. Allele-specific primers were designed by using the fact that a 3′ mismatch does not prime in a PCR at a specific annealing temperature (Sommer et al. 1992). Primers SETS1 and SETR1R were designed to specifically prime *Setaria* sp. ACCase sequences containing A or C at nucleotide position 5338, respectively. Those primers were used together with primers ACSA and ACSAR at a final concentration of 0.1 µM for each of the four primers. PCR amplifications were performed in 20 µl as described by Délye et al. (1997). A 1-µl aliquot of the supernatant of DNA extracts was used for PCR. The cycling program consisted of one denaturation step of 30 s at 95 °C, followed by 37 cycles of 10 s at 95 °C, 15 s at 59 °C and 30 s at 72 °C. Primers were designed to generate up to three distinct sizes of amplicons. Primers ACSA and SETR1R yielded a 448-bp fragment, primers SETS1 and ACSAR yielded a 677-bp fragment, and primers ACSA and ACSAR yielded a 1,087-bp fragment. Thus, DNA from plants in which both ACCase gene copies contain A at nucleotide position 5338 would yield two fragments of 677 and 1,087 bp, DNA from plants in which both ACCase gene copies contain C at nucleotide position 5338 would yield two fragments of 448 and 1,087 bp, and amplification from heterozygous plants would yield three fragments of 448, 677 and 1,087 bp.

Results

Structure of *S. italica* chloroplastic ACCase

A 7,630-bp nucleotide sequence was obtained after assembling the sequences of the fragments amplified using PCR and RACE-PCR. It was deposited in the GenBank database (Acc. No. AF294805). A coding sequence starting at nucleotide 243 encoded a polypeptide of 2,321 amino acids, with a calculated molecular mass of 256 kDa. The coding sequence ended with two consecutive TGA stop codons. When compared with eukaryotic-type ACCases, the amino acid sequence of the ACCase from *S. italica* showed 94% and 87% identity with the chloroplastic, eukaryotic-type ACCases from maize and wheat, respectively. The nucleotide coding sequence of the ACCase from *S. italica* showed 92% and 85% identity with the coding sequences of the

chloroplastic, eukaryotic-type ACCases from maize and wheat, respectively. Those sequences are the two only other complete sequences of chloroplastic, eukaryotic type ACCases from the Gramineae reported so far. The next sequence most similar to the ACCase from *S. italica* is the eukaryotic-type, cytosolic ACCase from wheat. Phylogenetic relationships of the 19 available, complete, eukaryotic-type ACCases indicated they could be divided into 5 major groups comprised of animal, fungal, diatom/diatom-like plastidic, plant chloroplastic and plant cytosolic enzymes, respectively. As shown in Fig. 1, the protein putatively encoded by the gene we cloned from *S. italica* was much closer to the plant eukaryotic-type, chloroplastic ACCase than to any other of the five groups, thus confirming we cloned the expected cDNA from *S. italica* encoding chloroplastic ACCase.

The main organisation of *S. italica* chloroplastic ACCase protein did not differ from those of other known eukaryotic-type ACCases. A 100-amino-acid region (amino acid positions 1–100) at the N-terminal end of the deduced protein contained a high number of hydroxylated and small, hydrophobic amino acids, typical of chloroplast transit peptides (Schleiff and Soll 2000). Although the sequences of transit peptides have been reported to be highly variable from one plant to another (Schleiff and Soll 2000), the amino acid sequence of this 100-amino-acid region in *S. italica* showed 89% identity with the transit peptide sequence of the chloroplastic ACCase from maize (Egli et al. 1995), thus strongly supporting this conclusion. The four most conserved amino acid regions among eukaryotic-type ACCases, the biotin-carboxylase domain, the biotin-carboxyl carrier domain and the β- and α- domains of the CT (Gornicki et al. 1994), are located at amino acid positions 136–633, 662–841, 1658–1707 and 1936–1969, respectively. Comparison with other ACCase sequences and searches for homologies in databases identified a putative ATP-binding site at position 312–331, within the biotin-carboxylase domain. A carboxybiotin-binding domain was found at amino acid position 1658–1707, within the β-subunit of the CT. An acetyl-CoA-binding site was identified within the α-subunit of the CT, at amino acid position 1946–1965. The highly conserved biotin-binding site K is located within the biotin-carboxyl carrier protein domain, at amino acid position 806.

Comparison of ACCase coding sequences from *Setaria* sp. lines sensitive or resistant to sethoxydim

One-hundred seedlings per *Setaria* sp. line investigated were assessed for sensitivity to sethoxydim. Seed bioassays showed that 100% of the seedlings from *S. viridis* line UM131 and from the *S. italica* introgression line D97-5a were resistant to sethoxydim, while 100% of the seedlings from all four other *Setaria* sp. lines were sensitive. Complete chloroplastic ACCase coding sequences were determined from each of the five *Setaria* sp. lines and compared with that from the *S. italica* parental line Shda1 (sensitive to sethoxydim). Only six nucleotide substitutions were recorded (Table 2). ACCase coding sequences from the *S. italica* parental line Shda1 and from the sethoxydim-sensitive *S. italica* introgression line D97-6a were identical. ACCase coding sequences from the *S. viridis* parental line UM131 and from the sethoxydim-resistant *S. italica* introgression line D97-5a were also identical, and displayed one synonymous and two non-synonymous mutations when compared with the sequence from line Shda1. ACCase coding sequences

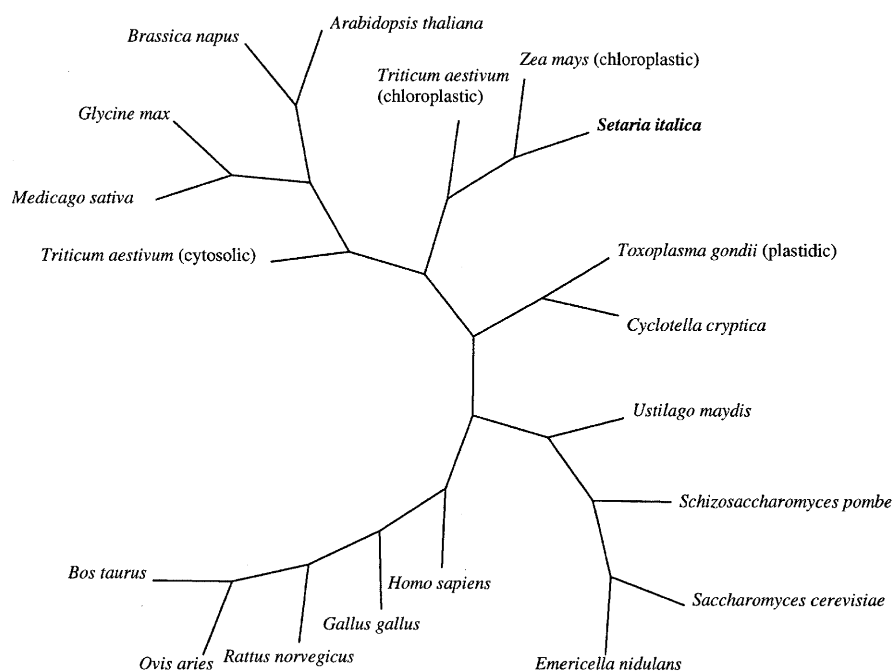

Fig. 1 Phylogenetic tree of eukaryotic-type ACCases. ACCAse sequences were obtained from GenBank. Predicted-protein-sequence accession numbers clockwise from *Triticum aestivum* (cytosolic) to *Bos taurus* are: U39321, L25042, L42814, T07920, D34630, AF029895, U19183, AF294805 (*S. italica*), AF157612, A48757, S49991, T38906, M92156, T30568, S41121, P11029, J03808, Q28559, AJ132890. Sequences were aligned using the Multalin software (Corpet 1988) with the Blosum 62 comparison table. The tree was generated by the program Protpars of the PHYLIP package (Phylogeny Inference Package, J. Felsenstein, University of Washington, Seattle, USA), using the parsimony method

Table 2 Nucleotide substitutions and amino acid changes found in *Setaria* sp. lines. ACCase sequences were compared with that from *S. italica* line Shda1 (GenBank Acc. No. AF294805)

Lines	Nucelotide position[a]	Substitution	Resulting amino acid substitution
S. viridis D90-19	315	A → G	Synonymous mutation (Val^{105})
S. viridis D90-19, D90-58	4383	C → T	Synonymous mutation (Asp^{1461})
S. viridis D90-19, D90-58, UM131; *S. italica* D97-5a	4564	G → A	Val^{1522} → Ile
S. viridis UM131; *S. italica* D97-5a	5338	A → C	Ile^{1780} → Leu
S. viridis D90-19, D90-58	5844	G → A	Synonymous mutation (Ala^{1948})
S. viridis D90-19, D90-58, UM131; *S. italica* D97-5a	6294	G → T	Synonymous mutation (Leu^{2098})

[a]The first nucleotide of the *S. italica* ACCase coding sequence is number 1

from sethoxydim-sensitive *S. viridis* lines D90-19 and D90-58 displayed one non-synonymous and four synonymous mutations, and one non-synonymous and three synonymous mutations, respectively, when compared with the sequence from line Shda1 (Table 2). Thus, *S. italica* introgression lines D97-5a and D97-6a had inherited the gene encoding chloroplastic ACCase from lines UM131 and Shda1, respectively.

A chloroplastic ACCase allele with one point mutation is a dominant gene for resistance to sethoxydim

Two non-synonymous mutations were found when comparing ACCase sequences from *S. viridis* lines and *S. italica* hybrid lines with that from the *S. italica* parental line Shda1. The first one is a valine-isoleucine substitution at amino acid position 1522. It has been found in all three *S. viridis* lines, and in the *S. italica* introgression line D97-5a that contains the gene encoding chloroplastic ACCase from the parental *S. viridis* line UM131. This substitution most likely reflects interspecific polymorphism. The second mutation is an isoleucine-leucine substitution at amino acid position 1780 that was found only in the parental *S. viridis* line UM131 and in the resistant *S. italica* introgression line D97-5a. The Leu^{1780} ACCase allele identified in *S. viridis* is thus the first mutation of a chloroplastic, eukaryotic-type ACCase responsible for resistance to sethoxydim identified so far.

An allele-specific PCR assay was developed to determine whether the presence of one or two Leu^{1780} ACCase allele(s) in the same plant was necessary to confer resistance to sethoxydim. Amplicons obtained from genomic DNA or cDNA from *Setaria* sp. using primer pair ACSA/ACSAR were of identical size (1,087 bp). The absence of introns within amplicons obtained from genomic DNA was confirmed by sequencing. The sensitivity of 20 seedlings from each of the *Setaria* sp. lines Shda1, UM131, D90-19, D90-58, D97-5a, and D97-6a and from two French populations of *S. viridis* collected in 1999 in Dijon (populations Ouges99 and Domaine99) to sethoxydim was determined using seed bioassay. The sensitivity of 220 seedlings obtained from various *S. italica* hybrid lines derived from the initial cross between UM131 and Shda1 was also determined. All 380 seedlings were analysed by allele-specific PCR (see Fig. 2 for illustration). All 20 seedlings from each of lines UM131 and D97-5a were resistant to sethoxydim and contained 2 copies of the Leu^{1780} ACCase allele. All 20 seedlings from each of lines Shda1, D90-19, D90-58, and D97-6a, and from populations Ouges99 and Domaine99 were sensitive to sethoxydim and contained 2 copies of the Ile^{1780} ACCase allele. Among the 220 *S. italica* hybrid seedlings, 134 were sensitive to sethoxydim and contained 2 copies of the Ile^{1780} ACCase allele. The 86 remaining seedlings were resistant to sethoxydim and consisted of 42 seedlings containing 2 copies of the Leu^{1780} ACCase allele, and of 44 seedlings containing 1 copy of the Ile^{1780} ACCase allele and 1 of the Leu^{1780} ACCase allele. No growth difference was observed between homozygous and heterozygous resistant seedlings. Thus, the mutant Leu^{1780} ACCase allele that confers resistance to sethoxydim is dominant.

Discussion

The isoleucine-leucine substitution responsible for resistance to sethoxydim in *S. viridis* is located within the 400-amino-acid region encompassing ACCase CT

Fig. 2 Allele-specific PCR analysis of 23 *Setaria* sp. seedlings using the four primers ACSA, SETS1, SETR1R and ACSAR. *Lanes 1, 4–8, 10, 11, 14, 16–18, 21* seedlings sensitive to sethoxydim, *lanes 2, 3, 9, 12, 13, 15, 19, 20, 22, 23* seedlings resistant to sethoxydim. Seedlings were issued from *S. italica* line Shda1 (*lane 1*), *S. viridis* line UM131 (*lane 2*), *S. italica* introgression lines D97-5a and D97-6a (*lanes 3, 4*), *S. viridis* lines D90-19, D90-58, Ouges99 and Domaine99 (*lanes 5–8*) and 15 various *S. italica* hybrid lines derived from the initial cross between UM131 and Shda1 (*lanes 9–23*). *Lane H* H_2O control (no DNA), *lane M* molecular weight marker (1 kb plus DNA ladder; Gibco BRL). The sizes of the amplified fragments are from top to bottom: 1,087, 677 and 448 bp

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                            1768                                               1819
S. italica (sensitive)      VVGK.EDGLGVENIHGSAAIASAYSRAYEETFTLTFVTGRTVGIGAYLARLGI
S. viridis (resistant)      VVGK.EDGLGVENLHGSAAIASAYSRAYEETFTLTFVTGRTVGIGAYLARLGI
T. aestivum (chloroplastic) VVGK.EDGLGVENIHGSAAIASAYSRAYEETFTLTFVTGRTVGIGAYLARLGI
Z. mays (chloroplastic)     VVGK.EDGLGVENIHGSAAIASAYSRAYEETFTLTFVTGRTVGIGAYLARLGI
A. sativa (chloroplastic)   VVGK.EDGLGVENIHGSAAIASAYSRAYEETFTLTFVSGRTVGIGAYLARLGI
T. aestivum (cytosolic)     IVGK.EDGLGCENLHGSGAIASAYSKAYRETFTLTFVTGRAIGIGAYLARLGM
H. vulgare (cytosolic)      IVGK.EDGLGCENLHGSGAIASAYSKAYRETFTLTFVTGRAIGIGAYLARLGM
A. thaliana                 IVGK.EDGIGVENLTGSGAIAGAYSKAYNETFTLTFVSGRTVGIGAYLARLGM
M. sativa                   IVGK.EDGLGVENLSGSGAIAGAYSRAYKETFTLTYVTGRTVGIGAYLARLGM
G. max                      IVGK.EDGLGVENLSGSGAIAGAYSRAYKETFTLTYVTGRTVGIGAYLARLGM
B. napus                    IVGK.EDGIGVENLTGSGAIAGAYSRGYRETFTLTFVSGRTVGIGAYLARLGM
B. taurus                   IIGK.EEGLGAENLRGSGMIAGESSLAYDEIITISLVTCRAIGIGAYLVRLGQ
R. norvegicus               IIGK.EEGLGAENLRGSGMIAGESSLAYDEIITISLVTCRAIGIGAYLVRLGQ
O. aries                    IIGK.EEGLGAENLRGSGMIAGESSLAYDEIITISLVTCRAIGIGAYLVRLGQ
G. gallus                   IIGK.EDGLGIENLRGSGMIAGESSLAYESIITINLVTCRAIGIGAYLVRLGQ
H. sapiens                  IIGK.DDGLGVENLRGSGMIAGESSLAYEEIVTISLVTCRAIGIGAYLVRLGQ
E. nidulans                 IIGA.KDGLGVECLKGSGLIAGATSRAYEDIFTITLVTCRSVGIGAYLVRLGQ
S. pombe                    IIGS.SEGLGVECLRGSGLIAGVTSRAYNDIFTCTLVTCRAVGIGAYLVRLGQ
U. maydis                   IIGKPQGGLGVECLSGSGLIAGETSRAKDQIFTATIITGRSVGIGAYLARLGE
C. cryptica                 IIGT.NEGIGVENLQGSGKIAGETSRAYDEIFTLSYVTGRSVGIGAYLVRLGQ
T. gondii                   IIGDPDKFIGVENLRGSGTIAGETSRAYDETFTLSYVTGRSVGIGAYIVRLAQ
                            + *+ ++++* *++ **+ **+  * ++ + +*   ++ *  *****+ ***
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Fig. 3 Alignment of the region of the CT domain of eukaryotic-type ACCases where an isoleucine-leucine substitution was found in *S. viridis* line UM131. The isoleucine residue is indicated in *bold*. ACCase sequences were obtained from GenBank [Acc. Nos. from top to bottom: AF294805 (*S. italica* and *S. viridis*), AF029895, U19183, AF072737 (partial sequence), U39321, X99102 (partial sequence), D34630, L25042, L42814, T07920, AJ132890, J03808, Q28559, P11029, S41121, T30568, T38906, S49991, A48757 and AF157612]. *Dots* indicate gaps. Identical residues are marked *, and conserved residues +. The positions of the first and last amino acids in the sequence from *S. italica* are indicated

domain that had been demonstrated to be involved in resistance to both APP and CHD herbicides in wheat (Nikolskaya et al. 1999). The isoleucine residue at position 1780 in *Setaria* sp. ACCase is conserved in all known eukaryotic-type, chloroplastic ACCases. In other eukaryotic-type ACCases (plant cytosolic ACCases, fungal and animal ACCases), which are much less sensitive to both APP and CHD herbicides, a leucine residue is found at this position (Fig. 3). Interestingly, the plastidic, eukaryotic-type ACCase from the parasitic protozoan parasite *Toxoplasma gondii*, which is sensitive to APP herbicides but resistant to CHD herbicides, including sethoxydim (Zuther et al. 1999), also has a leucine residue at a position corresponding to amino acid position 1780 in *S. viridis* (Fig. 3). The presence of an isoleucine residue in chloroplastic, eukaryotic-type ACCases instead of a leucine residue in cytosolic, eukaryotic-type ACCases is therefore most likely the molecular basis for sethoxydim selectivity towards plants other than members of the Gramineae.

Previous enzymatic studies demonstrated that the activities of the ACCases from the sethoxydim-resistant line UM131 and from a herbicide-sensitive *S. viridis* biotype were not significantly different (Shukla et al. 1997), probably because leucine and isoleucine have similar chemical structures. Thus, the conformational change in mutant ACCase from line UM131 is probably minor, although sufficient to prevent binding of sethoxydim.

Herbicide bioassays showed that *S. viridis* line UM131 had a high level of resistance to sethoxydim, but was moderately resistant to APP herbicides (Heap and Morrison 1996). This resistance pattern was transferred to the progeny of interspecific crosses performed between *S. viridis* line UM131 and *S. italica* line Shda1 (Wang and Darmency 1998). Enzymatic studies conduced by Shukla et al. (1997) also showed that altered chloroplastic ACCase from *S. viridis* line UM131 was highly resistant to sethoxydim and moderately resistant to APP herbicides. Thus, it is clear that another mutation(s) is (are) involved in resistance of *S. viridis* to APPs. This is in agreement with previous findings demonstrating that, although both classes of molecule inhibit ACCase, APPs and CHDs bind at different positions on the enzyme (reviewed in Gronwald 1991). Although *S. viridis* line UM131 is not highly resistant to APP herbicides, other accessions of this weed exhibit different resistance patterns (e.g. Marles et al. 1993) that may be investigated for target-enzyme alteration. Our cloning and sequencing the gene encoding ACCase in *S. viridis* and *S. italica* pave the way towards identification of other mutations responsible for various resistance patterns to ACCase-inhibiting herbicides. This will enable molecular tools such as our allele-specific PCR assay to be developed to monitor resistance alleles in the field. In contrast to herbicide-sensitivity bioassays, in which different genes of resistance responsible for similar resistance patterns cannot be discriminated, molecular markers enable a fast, accurate and non-destructive diagnosis of the genetic basis of resistance. Thus, they will be of great help in studying the dynamics of the appearance and propagation of resistance genes using population-genetics approaches.

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References

Alban C, Baldet P, Douce R (1994) Localization and characterization of two structurally different forms of acetyl-CoA carboxylase in young pea leaves, of which one is sensitive to aryloxyphenoxypropionate herbicides. Biochem J 300:557–565

Burton JD, Gronwald JW, Somers DA, Gegenbach BG, Wyse DL (1989) Inhibition of corn acetyl-coA carboxylase by cyclohexanedione and aryloxyphenoxypropionate herbicides. Pestic Biochem Physiol 34:76–85

Christopher JT, Holtum JAM (2000) Dicotyledons lacking the multisubunit form of the herbicide target enzyme acetyl coenzyme A carboxylase may be restricted to the family Geraniaceae. Aust J Plant Physiol 27:845–850

Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881–10890

Délye C, Laigret F, Corio-Costet MF (1997) A mutation in the 14α-demethylase gene of *Uncinula necator* that correlates with high levels of resistance to a sterol biosynthesis inhibitor. Appl Environ Microbiol 63:2966–2970

Devine MD, Shukla A (2000) Altered target sites as a mechanism of herbicide resistance. Crop Prot 19:881–889

Egli MA, Gegenbach BG, Gronwald JW, Somers DA, Wyse DL (1993) Characterization of maize acetyl-coenzyme A carboxylase. Plant Physiol 101:499–506

Egli MA, Lutz SM, Somers DA, Gegenbach BG (1995) A maize acetyl-coenzyme A carboxylase cDNA sequence. Plant Physiol 108:1299–1300

Evenson KJ, Gronwald JW, Wyse DL (1997) Isoforms of acetyl-coenzyme A carboxylase in *Lolium multiflorum*. Plant Physiol Biochem 35:265–272

Gornicki P, Podkowinski J, Scappino LA, DiMaio J, Ward E, Haselkorn R (1994) Wheat acetyl-coenzyme A carboxylase: cDNA and protein structure. Proc Natl Acad Sci USA 91:6860–6864

Gornicki P, Faris J, King I, Podkowinski J, Gill B, Haselkorn R (1997) Plastid-localised acetyl-CoA carboxylase of bread wheat is encoded by a single gene on each of the three ancestral chromosome sets. Proc Natl Acad Sci USA 94:14179–14184

Gronwald JW (1991) Lipid biosynthesis inhibitors. Weed Sci 39:435–449

Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol 39:101–138

Heap IM (2001) International survey of herbicide resistant weeds. Internet, available at www.weedscience.com

Heap IM, Morrison IN (1996) Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in green foxtail (*Setaria viridis*). Weed Sci 44:25–30

Konishi T, Shinohara K, Yamada K, Sasaki Y (1996) Acetyl-CoA carboxylase in higher plants: most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. Plant Cell Physiol 37:117–122

Marles MAS, Devine MD, Hall CJ (1993) Herbicide resistance in *Setaria viridis* conferred by a less sensitive form of acetyl coenzyme A carboxylase. Pestic Biochem Physiol 46:7–14

Menéndez J, De Prado R (1999) Characterization of two acetyl-CoA carboxylase isoforms in diclofop-methyl-resistant and -susceptible biotypes of *Alopecurus myosuroides*. Pestic Biochem Physiol 65:82–89

Nikolskaya T, Zagnitko O, Tevzadze G, Haselkorn R, Gornicki P (1999) Herbicide sensitivity determinant of wheat plastid acetyl-CoA carboxylase is located in a 400-amino acid fragment of the carboxyltransferase domain. Proc Natl Acad Sci USA 96:14647–14651

Ohlrogge JB, Jaworski JG (1997) Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 48:109–136

Podkowinski J, Sroga GE, Haselkorn R, Gornicki P (1996) Structure of a gene encoding a cytosolic acetyl-CoA carboxylase of hexaploid wheat. Proc Natl Acad Sci USA 93:1870–1874

Saini HS, Shepherd M, Henry RJ (1999) Microwave extraction of total genomic DNA from barley grains for use in PCR. J Inst Brew 105:185–190

Sasaki Y, Konishi T, Nagano Y (1995) The compartimentation of acetyl-coenzyme A carboxylase in plants. Plant Physiol 108:445–449

Schleiff E, Soll J (2000) Travelling of proteins through membranes: translocation into chloroplasts. Planta 211:449–456

Shukla A, Leach GE, Devine MD (1997) High-level resistance to sethoxydim conferred by an alteration in the target enzyme, acetyl-CoA carboxylase, in *Setaria faberi* and *Setaria viridis*. Plant Physiol Biochem 35:803–807

Sommer SS, Groszbar AR, Bottema CDK (1992) PCR amplification of specific alleles (PASA) is a general method for rapidly detecting known single base-pair changes. Biotechniques 12:82–87

Wang T, Darmency H (1997) Inheritance of sethoxydim resistance in foxtail millet, *Setaria italica* (L.) Beauv. Euphytica 94:69–73

Wang T, Darmency H (1998) Cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in foxtail millet (*Setaria italica*). Pestic Biochem Physiol 59:81–88

Zuther E, Johnson JJ, Haselkorn R, McLeod R, Gornicki P (1999) Growth of *Toxoplasma gondii* is inhibited by aryloxyphenoxypropionate herbicides targeting acetyl-CoA carboxylase. Proc Natl Acad Sci USA 96:13387–13392