Research Article

Received: 29 June 2018 Revised: 27 August 2018 Accepted article published: 6 September 2018 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.5197

Effect of *Ahasl1-1* and *Ahasl1-4* alleles on herbicide resistance and its associated dominance in sunflower

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Abstract

BACKGROUND: Acetohydroxyacid synthase large subunit 1 (*Ahasl1*) is a multiallelic *locus* involved in herbicide resistance in sunflower. *Ahasl1-1* and *Ahasl1-4* alleles harbor different point mutations that lead to different amino acid substitutions (Ala205Val and Trp574Leu, respectively). The objectives of this work were to evaluate the effect of these alleles at the enzymatic and whole-plant levels, and to determine the dominance relationships for imazapyr and metsulfuron-methyl herbicides.

RESULTS: Resistant near-isogenic lines showed significantly lower specific AHAS activity than susceptible near-isoline. However, kinetic studies indicated that mutations did not change AHAS pyruvate affinity. Dose–response for six near-isolines carrying different combinations of *Ahasl1-1* and *Ahasl1-4* alleles and two herbicides (imazapyr and metsulfuron-methyl) were evaluated at whole-plant and enzymatic levels. *Ahasl1-1* allele conferred moderate resistance to imazapyr and low resistance to metsulfuron-methyl. Conversely, *Ahasl1-4* allele endowed high levels of resistance for both herbicides. Dominance of resistance at whole-plant level showed a semi-dominant behavior among the alleles for both herbicides.

CONCLUSION: *Ahasl1-4* allele confers higher resistance levels than *Ahasl1-1* when evaluated with imazapyr and metsulfuron-methyl. Dominance estimations suggested that both parental lines should carry a resistance trait when developing hybrids.

Keywords: imidazolinones; sulfonylureas; herbicide-resistance; acetohydroxyacid synthase; *Helianthus annuus* L

1 INTRODUCTION

Acetohydroxyacid synthase (AHAS, EC 2.2.1.6) also known as acetolactate synthase (ALS) is the first enzyme in the biosynthesis of the branched chain amino acids valine, leucine and isoleucine.[1] AHAS is the target site of herbicides belonging to group B that includes structurally diverse chemical classes of molecules such as sulfonylureas (SU) and imidazolinones (IMI).[2] SU and IMI herbicides have been demonstrated to have a broad spectrum of weed control activity, flexibility in timing of application and low usage rates.[3,4] The availability of herbicide resistant hybrids would allow the use of these herbicides in a sunflower production system. IMI and SU-resistant plants have been discovered in sunflower, which permitted the development and commercialization of several herbicide-resistant traits.[5]

Three genes coding for the AHAS catalytic or large subunit (*Ahasl1*, *Ahasl2* and *Ahasl3*) were identified and characterized in sunflower.[6] A single base-pair change in the gene encoding the large subunit of AHAS1 (AHASL1) results in a herbicide resistant enzyme. Several different mutations in *Ahasl1* have been identified.[5] *Ahasl1-1* and *Ahasl1-4* are resistance alleles discovered in wild sunflower populations that confer moderate resistance to IMI and cross-resistance to different AHAS-inhibiting herbicides, respectively.[7,8] The amino acid changes identified at *Ahasl1-1* and *Ahasl1-4* alleles are Ala205Val and Trp574Leu (amino acid number in reference to AHAS sequence from *Arabidopsis thaliana*), respectively.[6,8]

Ahasl1-1 (also known as *Imr1*) represents the first commercial herbicide-resistant trait in sunflowers known as 'Imisun'.[5] The inheritance of Imisun is additively controlled by two genes, one of them is *Ahasl1-1* and the other a modifier or enhancer factor.[6,9] The modifier gene is involved in the non-target-site component of resistance of this trait.[10] *Ahasl1-4* allele endows high levels of resistance to at least four of the five families of herbicides targeting AHAS but this trait is not commercially available yet.[8]

Resistance-conferring amino acid substitutions are structural changes in AHAS that prevent or limit effective herbicide binding.[2] These resistance-conferring mutations at AHAS may

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cause changes on enzyme functionality and varies widely among species. Several works found that specific AHAS activity, kinetic parameters and/or feedback inhibition by the branched-chain amino acids were increased, decreased, and unchanged for different amino acid substitutions.[11–13] A previous study in wild sunflower showed no differences between AHAS extracted from resistant and susceptible biotypes.[7] Nonetheless, the impact of mutations on AHAS activity in cultivated sunflower is still unknown.

The combination of different herbicide-resistant alleles at the *Ahasl1 locus* of sunflower can be used to design specific resistance traits in the commercial F1 hybrid. Sala *et al.* found that *Ahasl1-3* allele that confers high levels of IMI-resistance showed dominance over *Ahasl1-1*.[14] From a practical perspective, this allows the possibility to produce commercial hybrids stacking both resistance alleles.

The objectives of this work were: (i) to evaluate the effect of *Ahasl1-1* and *Ahasl1-4* alleles at enzymatic level in absence of herbicide, (ii) to quantify the plant growth and AHAS activity response to different concentrations of IMI and SU herbicides in sunflower near-isolines carrying different combinations of the *Ahasl1-1* and *Ahasl1-4* alleles, and (iii) to estimate the dominance relationships between these alleles.

2 MATERIALS AND METHODS

2.1 Plant material and growth conditions

Six sunflower maintainer lines differing at *Ahasl1 locus* were used in this study. Three sunflower inbred near-isolines: susceptible (*ahasl1 ahasl1*), *Ahasl1-1* homozygous (*Ahasl1-1 Ahasl1-1*) and *Ahasl1-4* homozygous (*Ahasl1-4 Ahasl1-4*), and the hybrids derived from their crossing were developed by Nidera S.A.

Seeds were sown in plastic pots (4 cm wide, 5.5 cm high) filled with commercial perlite (3–8 mm; Multiquim SRL, Argentina) as described by Breccia *et al.*[15] Plants were grown under controlled conditions of temperature, photoperiod and light intensity (25 ± 2 °C, 16 h light and 100 µmol m^{-2} s^{-1} respectively). After 14-day incubation period plants presented two true leaves (V2 stage according to Schneiter and Miller)[16] which were excised and immediately used for image analysis or enzymatic assays.

2.2 AHAS kinetic assay

Kinetic studies were performed using partially purified AHAS extracted from young sunflower leaves following the procedure of Yu *et al.*[11] Pyruvate was omitted from the extraction and reaction buffers, and final concentrations of 1.25–100 mmol L^{-1} were used in the reaction mixtures. Both the H_2SO_4 and NaOH controls were used for each pyruvate concentration. Protein concentrations were determined by the method of Bradford.[17] Absorbance was measured spectrophotometrically at 530 nm using a spectrophotometer (Lambda Bio, PerkinElmer®). Specific AHAS activity and kinetic parameters were calculated from three independent extractions.

2.3 Herbicide response at whole-plant and AHAS activity levels

Two herbicides of AHAS inhibitors group were used: imazapyr (Clearsol®, BASF Argentina, WG 80% a.i.) and metsulfuron-methyl (Nufuron®, Nufarm Argentina, WG 60% a.i.) belonging to the IMI and SU chemical families, respectively.

The herbicide response at whole plant level was assessed through leaf area measurement. This variable was useful for the characterization of sunflower genotypes differing in herbicide resistance.[15] Twelve pots were placed in plastic trays and watered by capillarity with nutritive solution consisting in Murashige Skoog salts (1.1 g L^{-1})[18] with different concentrations of herbicide (0.001–100 µmol L^{-1} imazapyr or 0.001–1000 nmol L^{-1} metsulfuron-methyl). The controls for each genotype were watered with nutritive solution without herbicides. The total area of the first pair of leaves was measured through digital image analysis using *Tomato Analyzer* software.[19] The pot experiments were arranged as a randomized design with three repetitions for each combination of treatment and genotype. Each repetition consisted of 12 plants.

AHAS activity inhibition was determined following the procedure described by Yu *et al.* and Breccia *et al.*[20,21] Crude extract (100 µL) and the same volume of distilled water or different herbicide concentrations (0.1–10 000 µmol L^{-1} imazapyr or 0.1–10 000 nmol L^{-1} metsulfuron-methyl) were incubated at 37 °C for 60 min. The reaction was stopped with 3 mol L^{-1} H_2SO_4 (40 µL) and incubated at 60 °C for 15 min to convert acetolactate to acetoin. Afterwards, 0.5% m/v creatine (280 µL) and 5% m/v α-naphthol (280 µL) in 5 mol L^{-1} NaOH were added and the mixture incubated at 60 °C for 15 min. Acetoin was quantified by a modified colorimetric assay.[22] AHAS activity was calculated as the mean of three independent repetitions.

2.4 Estimation of dominance relationships

The dominance of herbicide resistance (DR) was estimated for *Ahasl1-4*, *Ahasl1-1* and *ahasl1* alleles for the two AHAS-inhibiting herbicides. DR was calculated according to Bourguet *et al.*: $DR = [\log(GR50_{RS}) - \log(GR50_{SS})]/[\log(GR50_{RR}) - \log(GR50_{SS})]$[23] where GR50 is the herbicide concentration required for 50% reduction of leaf area and RR represents the homozygous genotype for a resistance allele R (*Ahasl1-1* or *Ahasl1-4*), RS represents the heterozygous genotype and SS is homozygous for S allele (*ahasl1* or *Ahasl1-1* when comparing *Ahasl1-4* and *Ahasl1-1*). DR was also calculated for AHAS activity inhibition using I50 instead of GR50. Effective dominance (ED) was calculated using the formula: $ED = (X_{RS} - X_{SS})/(X_{RR} - X_{SS})$ where X is the phenotypic trait (leaf area or AHAS activity) at each herbicide concentration.[23] Following convention, the resistance allele was considered dominant in presence of herbicide when dominance estimation was equal to 1, semi-dominant when was 0.5, and recessive when approached 0.[24]

2.5 Statistical analyses

Statistical analyses were performed using R software.[25] Specific AHAS activity of non-treated plants was analyzed by one-way analysis of variance. Means were separated using Fisher's protected least significant difference (LSD) test at the 5% probability level. AHAS kinetic parameters were estimated for each homozygous near-isoline by fitting the data to the Michaelis–Menten equation: $v = Vmax_{app}\ S/(Km_{app} + S)$, where v is the AHAS reaction velocity, $Vmax_{app}$ is the maximal enzymatic velocity, Km_{app} is the Michaelis–Menten constant for pyruvate, and S is the pyruvate concentration. The *drc* package within R software was used.[26] Dose–response curves were adjusted to log-logistic model of three parameters: $y = d/[1 + (x/e)^b]$ where e (also known as GR50 or I50) denotes the herbicide dose (x) that inhibited plant response (y) by 50%; d reflects the response upper limit and b denotes the relative slope around e.[27] The response lower limit was considered equal to 0. The comparison of estimated parameters between genotypes was assessed with the *compParm* function of *drc* package.

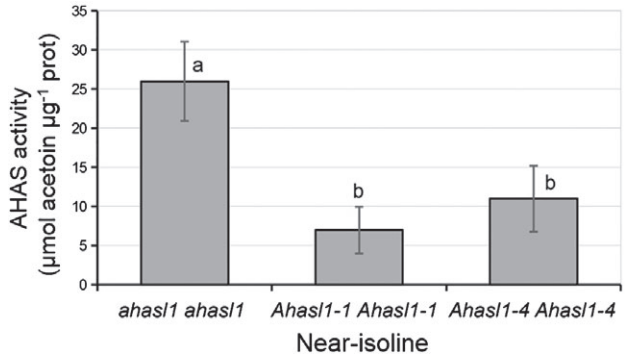

Figure 1. Total AHAS activity measured from partially purified enzyme extracts of three sunflower near-isolines that differ at *Ahasl1 locus*: susceptible (*ahasl1 ahasl1*), *Ahasl1-1* homozygous and *Ahasl1-4* homozygous near-isolines. Means with different letters are significantly different at $P < 0.05$.

Table 1. Apparent AHAS constant for pyruvate (Km_{app}) and apparent AHAS maximal velocity ($Vmax_{app}$) values of three near-isolines differing at *Ahasl1 locus*: susceptible (*ahasl1 ahasl1*), *Ahasl1-1* homozygous and *Ahasl1-4* homozygous near-isolines

Near-isoline *Ahasl1* genotype	Km_{app} (mmol L^{-1} pyruvate)	$Vmax_{app}$ ($\Delta abs\ h^{-1}$)
ahasl1 ahasl1	2.5 ± 0.2*a*	2.524 ± 0.046*a*
Ahasl1-1 Ahasl1-1	4.1 ± 2.1*a*	0.434 ± 0.054*c*
Ahasl1-4 Ahasl1-4	1.5 ± 0.3*a*	1.365 ± 0.045*b*

Different letters after means within the same column indicate significant differences at $P < 0.05$.

3 RESULTS

3.1 Effect of resistance alleles on AHAS kinetics

The effect of *Ahasl1-1* and *Ahasl1-4* alleles that lead to Ala205Val and Trp574Leu mutations in AHASL1 respectively, was studied at enzymatic level. Total AHAS activity extracted from both homozygous resistant near-isolines (*Ahasl1-1 Ahasl1-1* and *Ahasl1-4 Ahasl1-4*) was significantly lower than total AHAS activity from susceptible near-isoline (Fig. 1). The maximum rate of enzyme activity ($Vmax_{app}$) and the Michaelis constant for pyruvate (Km_{app}) were estimated (Table 1). $Vmax_{app}$ values of the resistant AHAS were significantly lower than susceptible (wild-type). Km_{app} values did not differ among near-isolines.

3.2 Herbicide response at whole-plant and AHAS activity levels

The response to imazapyr and metsulfuron-methyl herbicides was evaluated in plants carrying the resistance alleles *Ahasl1-1* and *Ahasl1-4* in homozygous, heterozygous and heterozygous stacked states. Dose–response curves of the six near-isolines for imazapyr and metsulfuron-methyl are shown in Figure 2. *Ahasl1-4* homozygous near-isoline presented the highest level of resistance to both herbicides (Table 2). Estimates of the herbicide concentration required for 50% reduction of leaf area (GR50) for imazapyr and metsulfuron-methyl were >260- and >1400-fold that of the susceptible near-isoline, respectively. GR50 values of the *Ahasl1-1* homozygous near-isoline were 100- and 13-fold higher than susceptible near-isoline for imazapyr and metsulfuron-methyl, respectively (Table 3).

The imazapyr resistance level of heterozygous near-isolines was greater for the stacked heterozygous near-isoline (*Ahasl1-4 Ahasl1-1*) with GR50 values >200-fold than the susceptible near-isoline (Table 2). Heterozygous *Ahasl1-4 ahasl1* and *Ahasl1-1 ahasl1* near-isolines showed GR50 values for imazapyr of about 20 times greater than the susceptible. Plants carrying the *Ahasl1-4* allele in heterozygous or heterozygous stacked state (*Ahasl1-4 ahasl1* and *Ahasl1-4 Ahasl1-1*) presented GR50 values >100-fold that of susceptible near-isoline for metsulfuron-methyl. In contrast, *Ahasl1-1* heterozygous near-isoline (*Ahasl1-1 ahasl1*) showed only three times greater GR50 values than the susceptible near-isoline for this herbicide.

Herbicide concentration required for 50% reduction of AHAS activity (I50) also varied among near-isolines (Table 2). I50 values of *Ahasl1-4* homozygous were almost 20-fold that of the susceptible near-isoline for both herbicides (Table 4). Estimates of I50 for imazapyr and metsulfuron-methyl in *Ahasl1-1* homozygous near-isoline were 3- and 1.5-fold that of the susceptible near-isoline, respectively. Near-isolines carrying the *Ahasl1-4* allele in heterozygous and heterozygous stacked states showed I50 values for imazapyr and metsulfuron-methyl >2.5-fold that of the susceptible near-isoline. Estimates of I50 for imazapyr and metsulfuron-methyl in *Ahasl1-1* heterozygous near-isoline were <2.2-fold that of the susceptible near-isoline.

3.3 Estimation of dominance of resistance and effective dominance

Dominance of resistance was calculated considering the response at both whole-plant and AHAS activity levels. The resistance alleles were almost semi-dominant in the presence of imazapyr or metsulfuron-methyl when considering GR50 values (Table 3). Similar dominance estimations were obtained at enzymatic level for *Ahasl1-4* and *Ahasl1-1* over *ahasl1* (Table 4). By the contrary, the *Ahasl1-4* allele was almost recessive over *Ahasl1-1* at enzymatic level for both herbicides.

Dominance relationships were also calculated as a function of herbicide concentration. Effective dominance (ED) for leaf area varied from dominance to near recessivity with increasing concentrations of herbicides for resistance alleles, except for *Ahasl1-4* over *Ahasl1-1* that presented a constant semi-dominance for imazapyr, and *Ahasl1-1* over *ahasl1* that showed a reduction from semi-dominance to recessivity for metsulfuron-methyl (Fig. 3(A) and (B)).

At the enzymatic activity level, *Ahasl1-4* allele showed a semi-dominant behavior over the susceptible allele that decreased with increasing herbicide concentrations (Fig. 3(C) and (D)). ED values for *Ahasl1-1* allele over susceptible also decreased with increasing herbicide concentration but in a different ED range for each herbicide, dominant to semi-dominant for imazapyr and near recessive for metsulfuron-methyl. On the other hand, the *Ahasl1-4* allele was almost recessive over *Ahasl1-1* at various herbicide concentrations.

4 DISCUSSION

Specific activity of AHAS from homozygous *Ahasl1-1Ahasl1-1* and *Ahasl1-4 Ahasl1-4* resistant near-isolines was less than 42% of the specific activity from the susceptible near-isoline (Fig. 1), suggesting that the resistance allele has detrimental effects on enzyme function, expression, or stability. The three near-isolines showed no significant differences in their AHAS Km_{app} (Table 1) suggesting pyruvate binding is not affected by substitutions at Ala205 and

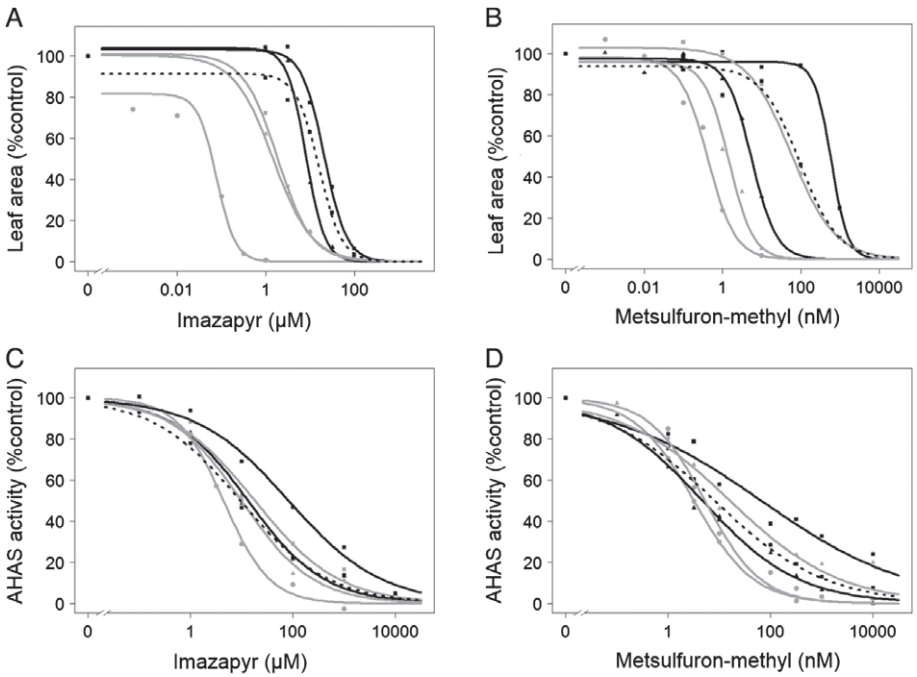

Figure 2. Effect of two AHAS-inhibiting herbicides on leaf area (A and B) and AHAS activity (C and D) of six sunflower near-isolines. Estimated dose–response curves and means are shown for near-isolines differing at *Ahasl1 locus*: susceptible (—●—, *ahasl1 ahasl1*), *Ahasl1-1* homozygous (—▲—, *Ahasl1-1 Ahasl1-1)*, *Ahasl1-1* heterozygous (—△—, *Ahasl1-1 ahasl1)*, *Ahasl1-4* homozygous (—■—, *Ahasl1-4 Ahasl1-4)*, *Ahasl1-4* heterozygous (—□—, *Ahasl1-4 ahasl1)*, and *Ahasl1-4 Ahasl1-1* stacked heterozygous (-■-).

Table 2. Herbicide concentration required for 50% reduction of leaf area (GR50) or AHAS activity (I50) of six sunflower near-isolines differing at *Ahasl1 locus*

Near-isoline*Ahasl1* genotype	Imazapyr		Metsulfuron-methyl	
	GR50 (μmol L^{-1})	I50 (μmol L^{-1})	GR50 (nmol L^{-1})	I50 (nmol L^{-1})
Ahasl1-4 Ahasl1-4	20.76 ± 4.14*a*	82.91 ± 13.50*a*	596.29 ± 331.11*a*	66.04 ± 17.05*a*
Ahasl1-1 Ahasl1-1	8.02 ± 1.28*b*	13.30 ± 2.37*b*	5.57 ± 1.02*b*	4.71 ± 0.89*c*
ahasl1 ahasl1	0.08 ± 0.02*d*	4.39 ± 0.49*d*	0.42 ± 0.08*d*	3.19 ± 0.47*d*
Ahasl1-4 Ahasl1-1	15.87 ± 3.74*ab*	9.53 ± 1.38*bc*	95.34 ± 31.45*a*	8.19 ± 1.76*b*
Ahasl1-4 ahasl1	1.85 ± 0.53*c*	18.15 ± 2.95*b*	56.62 ± 20.21*a*	16.29 ± 3.32*b*
Ahasl1-1 ahasl1	1.46 ± 0.48*c*	9.88 ± 1.50*bc*	1.45 ± 0.28*c*	5.22 ± 0.73*c*

Different letters after means within the same column indicate significant differences at $P < 0.05$.

Trp574. These results are in agreement with the location of the mutations, not at the active site but within the substrate-access channel.[28] Moreover, AHAS kinetic studies with sunflower and several other species have shown similar Km_{app} values for resistant and susceptible genotypes.[7,12,29–31]

The $Vmax_{app}$ was higher in the susceptible near-isoline compared to the resistant ones (Table 1). Since Km_{app} estimates were similar, this would suggest that wild-type AHAS can achieve a higher rate of pyruvate formation at Km_{app}, and thus, a higher catalytic efficiency. Accordingly, levels of extractable AHAS activity was also higher in the susceptible near-isolines (Fig. 1). Several works showed that herbicide resistance-endowing AHAS gene mutations result in decreased AHAS activity in dicots.[30,32–34] Increased or unchanged AHAS activity has been observed for a number of resistance mutations in other species.[11,12,33] The difference in AHAS activity observed between resistant and susceptible genotypes is therefore likely to be related to specific resistance mutations in different plant species, frequency of resistance alleles in the resistant weed population, and the genetic background of the genotypes under comparison. In this study, the near-isogenic lines provide appropriate materials for studying the specific effects of the resistance alleles in the absence of confounding background effects.[30,35]

Bourguet and Raymond defined a threshold activity as the maximum decrease of the enzyme activity that can be tolerated without affecting the phenotype.[36] This threshold or safety margin was documented for AHAS enzyme to be much lower than 50%.[36] Similar results were found in the present work, since a significant reduction in specific activity of AHAS from homozygous resistant near-isolines were observed without affecting plant growth and yield in lines and hybrids (Bulos M, 2016, pers. comm.).

Cross-resistance pattern endowed by a given AHAS resistance mutation is dependent on specific mutations, AHAS inhibitor chemical groups and sometimes species.[37] *Ahasl1-1* allele conferred moderate resistance to IMI herbicides and low resistance levels to SU (Table 2). This cross-resistance pattern was also

Table 3. Herbicide resistance ratios (RR/SS) and dominance of herbicide resistance (DR) of the *Ahasl1-1* and *Ahasl1-4* alleles for two AHAS-inhibiting herbicides: imazapyr (IMI) and metsulfuron-methyl (SU)

Alleles (R/S)	Herbicide	RR/SS	Comparison RS - SS	DR
Ahasl1-4/ahasl1	IMI	265.7 ± 80.1	*Ahasl1-4ahasl1 > ahasl1 ahasl1*	0.57
	SU	1415.6 ± 832.2		0.68
Ahasl1-1/ahasl1	IMI	102.7 ± 28.4	*Ahasl1-1 ahasl1 > ahasl1 ahasl1*	0.63
	SU	13.2 ± 3.5		0.48
Ahasl1-4/Ahasl1-1	IMI	2.6 ± 0.7	*Ahasl1-4 Ahasl1-1 > Ahasl1-1Ahasl1-1*	0.72
	SU	107.0 ± 62.6		0.61

RR/SS and DR were calculated with estimated GR50 (herbicide concentration required for 50% reduction of leaf area)

Table 4. Resistance ratios (RR/SS) and dominance of herbicide resistance (DR) of the *Ahasl1-1* and *Ahasl1-4* alleles for two AHAS-inhibiting herbicides: imazapyr (IMI) and metsulfuron-methyl (SU)

Alleles (R/S)	Herbicide	RR/SS	Comparison RS - SS	DR
Ahasl1-4/ahasl1	IMI	18.9 ± 3.7	*Ahasl1-4ahasl1 > ahasl1 ahasl1*	0.48
	SU	20.7 ± 6.2		0.54
Ahasl1-1/ahasl1	IMI	3.0 ± 0.6	*Ahasl1-1 ahasl1 > ahasl1 ahasl1*	0.73
	SU	1.5 ± 0.4		-- [a]
Ahasl1-4/Ahasl1-1	IMI	6.2 ± 1.5	*Ahasl1-4 Ahasl1-1 = Ahasl1-1Ahasl1-1*	−0.18
	SU	14.0 ± 4.5	*Ahasl1-4 Ahasl1-1 > Ahasl1-1Ahasl1-1*	0.21

RR/SS and DR were calculated with estimated I50 (herbicide concentration required for 50% reduction of AHAS activity).
[a] Resistant allele did not confer resistance advantage (RR/SS < 2) so that DR was not calculated.

observed for wild sunflower resistant biotypes.[38,39] Conversely, *Ahasl1-4* allele endowed high levels of resistance for both IMI and SU herbicides. In fact, Trp574Leu mutation was shown to provide cross-resistance to five chemical classes in sunflower and other species.[8,40–42]

The AHAS activity response to herbicides was not as sensitive as the plant response which showed higher resistance ratios (Tables 3 and 4). Similar results were found by previous works in weed species.[12,43–45] The lower levels of herbicide resistance observed at enzymatic level could be attributed to the inhibition of the susceptible AHAS isoforms coded by paralogous genes. *Ahasl2* was shown to be expressed in the first pair of true leaves and could be responsible for the dilution of the resistance in the pool of AHAS enzymes.[21,46]

The response of stacked heterozygous near-isoline at enzymatic level showed a different trend than at whole-plant level for both tested herbicides. Consequently, dominance estimations at enzymatic level were also different (Fig. 3, Table 3 and 4). *Ahasl1-4* allele was mostly semi-dominant compared to *Ahasl1-1* at the phenotypic level, even though it was recessive at the enzymatic level. This discrepancy can be explained taking account the protein structure of the AHAS catalytic subunits and the threshold or safety margin of the enzyme. Crystallographic AHAS from plants consist of four catalytic subunits.[28] The active site of the enzyme is at the interface of two monomers; hence the minimal requirement for AHAS activity is a dimer.[2] *Ahasl1-4 Ahasl1-1* stacked heterozygous plants carry two different types of AHAS catalytic subunit that can be combined in three types of AHAS protein: one composed by AHASL1-1 subunits, another composed by AHASL1-4 subunits and the third one composed by a mix of both types of subunits (heterodimer). The enzymatic inhibition reflects, on average, the behavior of these three types of enzymes. If the heterodimer was more inhibited than the resistant homodimer, the resistance would be much lower in the heterozygous compared to the resistant homozygous near-isoline. Similar results were found in heterozygous rice plants.[47] The recessivity of herbicide resistance at the enzymatic level could be attributed to the heterodimer formation and its inhibition behavior. On the other hand, the deleterious effect of the stacked heterozygous in the AHAS pool is not translated at phenotypic level and this observation could be explained considering the safety margin of the AHAS as previously discussed.

The results of the present work showed that effective dominance at whole-plant and enzymatic levels varied from completely dominant to recessive, depending on the resistance allele and the type and concentration of herbicide (Fig. 3). These results are in agreement with previous findings for herbicide resistant alleles in *A. thaliana* and sunflower.[14,24] Herbicide resistance for aerial growth was almost semi-dominant in the presence of imazapyr or metsulfuron-methyl (Table 3). Similar results were observed for sugarbeet, chicory and sunflower.[9,48,49] A practical implication of the intermediate response of heterozygotes is the lower margin of crop safety to postemergence use of IMI or SU herbicides. For this reason, both parental lines should carry the resistance trait when developing commercial hybrids. On the other hand, other practical aspects should be optimized for specific growing conditions such as application date and mix combinations of active ingredients and adjuvants.[50]

5 CONCLUSIONS

The broad spectrum and high resistance level of *Ahasl1-4* would lead to the development of a new versatile technology. Independently of the type of AHAS-inhibitor herbicide applied over the top of the crop, the cross-tolerance of *Ahasl1-4* could allow sunflower hybrids carrying this allele to cope with the soil residues of other

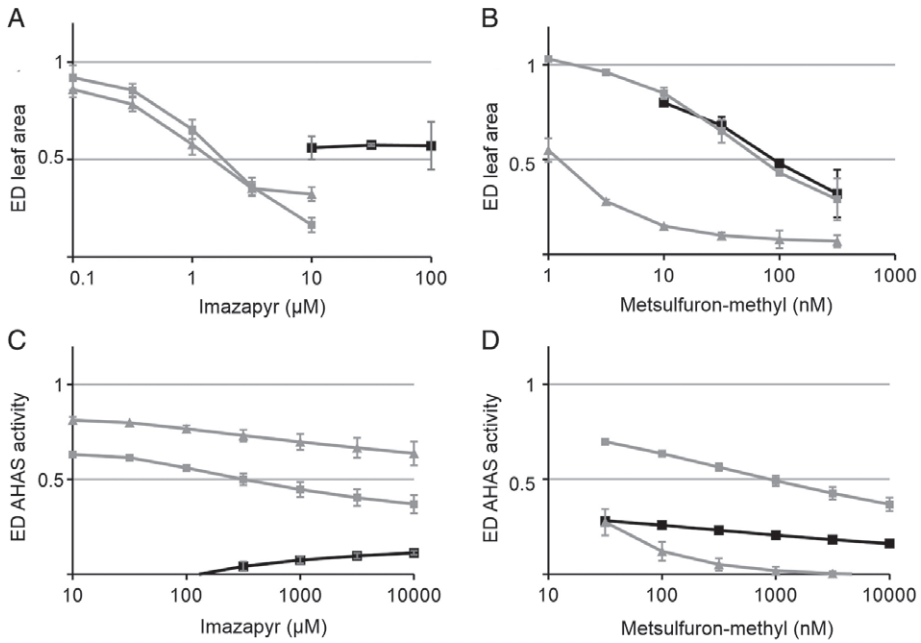

Figure 3. Effective dominance (ED) for leaf area (A and B) and AHAS activity (B and C) for two AHAS-inhibiting herbicides. ED was calculated at herbicide concentrations for which the resistant allele conferred a resistance advantage (resistance ratio > 2). *Ahasl1-4* over the susceptible (*ahasl1*) (—■—), *Ahasl1-1* over the susceptible (*ahasl1*) (—▲—), and *Ahasl1-4* over *Ahasl1-1* (—■—).

types of AHAS-inhibiting herbicides from the fallow or the previous crop. Moreover, this allele also allows the possibility to apply the appropriate AHAS-inhibiting herbicide according to the weed species composition occurring in each sunflower field.

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