Transgenic Res (2012) 21:1255–1264
DOI 10.1007/s11248-012-9597-z

ORIGINAL PAPER

Comparability of imazapyr-resistant Arabidopsis created by transgenesis and mutagenesis

Jaimie Schnell · Hélène Labbé · Nik Kovinich · Yuzuki Manabe · Brian Miki

Received: 27 September 2011 / Accepted: 3 February 2012 / Published online: 21 March 2012

Abstract The Arabidopsis *CSR1* gene codes for the enzyme acetohydroxyacid synthase (AHAS, EC 2.2.1.6), also known as acetolactate synthase, which catalyzes the first step in branched-chain amino acid biosynthesis. It is inhibited by several classes of herbicides, including the imidazolinone herbicides, such as imazapyr; however, a substitution mutation in *csr1-2* (Ser-653-Asn) confers selective resistance to the imidazolinones. The transcriptome of *csr1-2* seedlings grown in the presence of imazapyr has been shown in a previous study (Manabe in Plant Cell Physiol 48:1340–1358, 2007) to be identical to that of wild-type seedlings indicating that AHAS is the sole target of imazapyr and that the mutation is not associated with pleiotropic effects detectable by transcriptome analysis. In this study, a lethal null mutant, *csr1-7*, created by a T-DNA insertion into the *CSR1* gene was complemented with a randomly-inserted *35S/CSR1-2/NOS* transgene in a subsequent genetic transformation event. A comparison of the *csr1-2* substitution mutant with the transgenic lines revealed that all were resistant to imazapyr; however, the transgenic lines yielded significantly higher levels of resistance and greater biomass accumulation in the presence of imazapyr. Microarray analysis revealed few differences in their transcriptomes. The most notable was a sevenfold to tenfold elevation in the *CSR1-2* transcript level. The data indicate that transgenesis did not create significant unintended pleiotropic effects on gene expression and that the mutant and transgenic lines were highly similar, except for the level of herbicide resistance.

Keywords Mutagenesis · Transgenesis · Transcriptome · Unintended effects · Acetohydroxyacid synthase · Acetolactate synthase

Electronic supplementary material The online version of this article (doi:10.1007/s11248-012-9597-z) contains supplementary material, which is available to authorized users.

J. Schnell · H. Labbé · N. Kovinich · Y. Manabe · B. Miki
Bioproducts and Bioprocesses, Research Branch, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada

Present Address:
J. Schnell (✉)
Plant and Biotechnology Risk Assessment Unit, Canadian Food Inspection Agency, 1400 Merivale Road, Ottawa, ON K1A 0Y9, Canada
e-mail: Jaimie.Schnell@inspection.gc.ca

Present Address:
Y. Manabe
Feedstocks Division, Joint BioEnergy Institute, Lawrence Berkely National Laboratory, 1 Cyclotron Road, MS 978R4121, Berkeley 94720-8205, CA, USA

Introduction

Transgenesis provides an important experimental strategy for generating knowledge on the functions and potential uses of cloned plant genes for

agriculture. Growing pressures to create new crops that are better suited for sustainable production (Park et al. 2011) demands an examination of the regulatory frameworks that govern the uptake of transgenic crops into commercial production (Smyth and McHughen 2008; McHughen and Smyth 2008). A significant barrier to the commercialization of transgenic crops is the diversity of regulatory policies and the limited extent of scientific data supporting them (McHughen 2007). For example, induced mutations have generally been used in plant breeding without the same scrutiny imposed on transgenic crops. Consequently mutation strategies are being encouraged and the use of transgenics discouraged (Waugh et al. 2006; Parry et al. 2009). Yet, recent studies reveal that mutagenesis can create more transcriptional changes in rice than transgenesis (Batista et al. 2008) and that variation in transcriptomes, proteomes or metabolomes of many crops is lower in transgenic crops than among conventionally bred varieties already in production (Kogel et al. 2010; Baudo et al. 2006; Lehesranta et al. 2005). Transgenesis also offers a wider range of options in the modification of a trait as regulatory and/or functional domains within genes can be separately modified with a greater variety of sequences. Avoidance of transgenesis may undermine the full potential of cloned genes to provide the novel traits needed for the production of foods, feeds and agricultural products within the more restrictive environments of the future.

The regulatory system in Canada is unique in that it examines the novelty of traits in new crop varieties and not the process used to generate them (Smyth and McHughen 2008). A crop variety with a novel trait, such as herbicide resistance, would undergo the same regulatory process whether generated by mutagenesis or transgenesis (Smyth and McHughen 2008). In other countries the use of transgenesis would initiate a more rigorous regulatory process than for mutagenesis. The rationale for the Canadian approach is to capture a scientific evaluation of the biosafety of a novel trait without bias towards or against new methodologies. Comprehensive data on the effects of trangenesis on compositional variation and risk is being generated using non-targeted profiling technologies (Kuiper et al. 2003; Davies 2010). This information complements but does not replace the traditional targeted approaches for examining the performance and composition of new crop varieties and provides perspective on the impact of transgenesis on biosafety and risk (Cellini et al. 2004; Parrott et al. 2010).

Here, we compare Arabidopsis plants with the same novel trait, i.e. imidazolinone resistance, produced by different genetic processes, i.e. mutagenesis and transgenesis. The target gene, *CSR1,* codes for the catalytic subunit of acetohydroxyacid synthase [AHAS, EC 2.2.1.6; a. k. a. acetolactate synthase (ALS)], a holoenzyme complex consisting of tetrameric subunits that catalyzes the first common step of the branched chain amino acid (BCAA) pathway (McCourt et al. 2006). AHAS inhibitors include slow-acting, systemic herbicides that can be used as soil-applied or post-emergent herbicides because they are absorbed by roots and foliage. Currently, five structurally distinct classes of herbicide are known to inhibit AHAS: imidazolinones, sulfonylureas, triazolopyridines, sulfonylaminocarbonyltriazolinones and pyrimidyl (oxy/thio) benzoates (McCourt and Duggleby 2006). The imidazolinone herbicides are used extensively in agriculture in combination with imidazolinone-tolerant Clearfield© crops (Tan et al. 2005), which exhibit resistance due to specific mutations in the gene coding for the AHAS catalytic subunit (Duggleby et al. 2008). AHAS mutations responsible for selective resistance to the imidazolinones have been mapped and include *csr1-2* (Ser-653-Asn), *csr1-5* (Ala-122-Thr) and *csr1-6* (Ala-205-Val) (Duggleby et al. 2008). The point mutations responsible for herbicide resistance function by creating amino acid substitutions that decrease the binding affinity of the herbicide to the substrate-access channel of the enzyme so that the herbicides can no longer block the enzyme's active site (Duggleby et al. 2008). Catalytic activity is not altered in the herbicide-resistant mutants, as the herbicide-binding domain is distinct from the active site (McCourt et al. 2006).

A comparison of the Arabidopsis substitution mutant *csr1-2* and wild-type plants using microarray analysis has revealed the complete absence of pleiotropic effects attributable to the mutation at the transcriptional level revealing that imidazolinones act specifically on AHAS as their sole target (Manabe et al. 2007). We now extend this study to transgenic plants in which the *CSR1-2* gene functionally replaces the native *CSR1* gene and show once again the absence of significant unanticipated pleiotropic effects under conditions that elevate the level of herbicide resistance and biomass accumulation. The study illustrates the

high similarity of a mutant and transgenic plant with the same novel trait.

Materials and methods

Plant materials and selection conditions

Growth conditions and imidazolinone treatments of *Arabidopsis thaliana* (ecotype: Col-0) plants were conducted as previously described (Manabe et al. 2007). 0.8% (w/v) and 1.2% Agar (Sigma, MO, USA) are used in place of 0.6% and 1.2% PHYTOAGAR as production of PHYTOAGAR had been discontinued. The reverse genetic database of the Salk Institute Genomic Analysis Laboratory (http://signal.salk.edu/cgi-bin/tdnaexpress) was searched for putative loss-of-function mutants of *CSR1* (At3g48560). Seeds of *csr1-2*^{D} and T-DNA insertional lines, *csr1-7* (SAIL_910_E06; CS877955) and *csr1-8* (GABI_562B05; N453873), were obtained from The Arabidopsis Biological Resource Center [(ABRC) Columbus, OH, USA]. T-DNA insertional mutants, *csr1-7* and *csr1-8*, were isolated from SAIL (Sessions et al. 2002) and GABI-KAT (Rosso et al. 2003) collections, respectively. Transformation vectors used in generating *csr1-7* and *csr1-8* are pDAP101, which carry the glufosinate-resistant (BAR) gene, and pAC161, which carries sulfadiazine-resistant (SUL1) gene, respectively. For selection, *csr1-7* and *csr1-8* were sown on MS-Agar medium supplemented with 25 mg/L analytical standard glufosinate-ammonium (Riedel-de Haën, Seelze, Germany) or with 75 mg/mL sulfadiazine (Sigma), respectively.

Plants were grown to maturity in a Conviron growth cabinet set to a temperature of 21°C and a 16 h day length. One to two week old seedlings were transplanted to 5 inch pots at a density of 5–6 plants per pots. These were fertilized 1 time per week with a solution of 20-20-20 general purpose fertilizer.

Genetic analysis

Genomic DNA was isolated from leaf tissue using the REDExtract-N-Amp Plant PCR kit (Sigma). PCR was also done using the REDExtract-N-Amp Plant PCR kit with primers at a final concentration of 0.3 μM. PCR cycling conditions were one cycle at 95°C for 3 min, 40 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min, and then a final extension at 72°C for 10 min. For amplification with the 8409 and SAIL_LP primer pair, an annealing temperature of 59°C was used instead of 53°C. The primers used in this study are: SAIL_RP: 5′-GTGCATCAATGGAGATTCACC-3′; SAIL_LP: 5′-CTTCCCCAAACGTCTTAAAGC-3′; 8409: 5′-ATATTGACCATCATACTCATTGC-3′; pDAP101-LB: 5′-TTCATAACCAATCTCGATACAC-3′. The position of each primer is shown in Online Resource 1.

Transformation

The coding region of the *CSR1-2* gene was synthesized (GenScript USA Inc.Piscataway, NJ, USA) and inserted into pCAMBIA1300 (http://www.cambia.org). The vector was transferred to *Agrobacterium tumefaciens* GV3101 and Agrobacterium-mediated transformation was accomplished using the floral dip method (Clough and Bent 1998). Heterozygous *CSR1/CSR1-7* plants were grown to the budding/flowering stage (6–8 weeks old) and inoculated with 500 mL Agrobacterium cultures grown to a cell density of 1.2–1.5 at OD_{600}. After centrifugation the bacteria were collected and resuspended in 300 mL of ½ strength MS media containing 5% sucrose. Silwet L-77 ("Vac-In-Stuff", Lehle Seeds, P.O. Box 2366, Round Rock Tx. 78680 USA) was added just prior to dipping at a concentration of 0.02% w/v. Mature flowers were removed before dipping and the infiltration treatment was maintained for 30 s. Following the treatment, the pots were placed on their sides, covered loosely with plastic wrap and placed in the dark for 24 h. The plants were then returned to the growth cabinet set to a temperature of 21°C and a 16 h day length until seed set.

Heterozygous *CSR1/CSR1-7* plants were essential for genetic transformation because the homozygous *CSR1-7/CSR1-7* genotype was lethal. Complementation and screening for segregants using PRC primers (Online Resource 1) allowed the recovery of viable homozygous *CSR1-7/CSR1-7* plants carrying the *CSR1-2* transgene (Online Resource 1).

Microarray analysis

For microarray analysis seedlings were grown in culture as above in the presence of 100 μg/L imazapyr for 2 weeks. Three separate biological samples consisting of 50–100 mg of fresh tissue consisting of the

shoots were collected as described by Manabe et al. (2007) and RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, CA, USA). Probe preparation, hybridization and analysis were carried out as previously described (Abdeen et al. 2010) by Affymetrix for the ATH1 GeneChip (www.affymetrix.com) at the Winnipeg Research Centre labs of Agriculture and Agri-Food Canada.

Results

Loss-of-function mutants reveal that *CSR1* is an essential housekeeping gene

To be sure that AHAS is an essential housekeeping gene we conducted a search for putative loss-of-function mutants of *CSR1*. Two independent and different T-DNA insertional alleles, *csr1-7* and *csr1-8,* were obtained and used for further analysis. The *csr1-7* and *csr1-8* mutations are at different locations within the only exon of *CSR1* (Online Resource 1). The *csr1-7* and *csr1-8* mutants were sown on MS-agar medium supplemented with 25 mg/L glufosinate-ammonium or with 75 mg/mL sulfadiazine, respectively, for selection of the mutant alleles. The genotypes were assessed by PCR using the primers shown in Online Resource 1. Plants that were susceptible on selection possessed the wild-type *CSR1/CSR1* genotype and all resistant plants were heterozygous *CSR1/CSR1-7* or *CSR1/CSR1-8* genotypes with a wild-type and a mutant allele. There were no homozygous mutants with the *CSR1-7/CSR1-7* or *CSR1-8/CSR1-8* genotypes found among progeny derived from selfed, heterozygous mutant plants (Table 1) indicating that these were lethal genotypes. This finding is consistent with observations that incomplete silencing of *CSR1* transcriptional activity by either anti-sense suppression or herbicide treatment results in plants which are almost non-viable (Höfgen et al. 1995).

Among the progeny of selfed heterozygous mutant plants the ratios of heterozygous mutant plants to wild-type plants were lower than expected. As shown in Table 1, 22% of the selected plants were *CSR1/CSR1-7* and 42% were *CSR1/CSR1-8*. As both are below the expected 67% (Table 1) there appeared to be reduced fitness among the heterozygous plants; however, plants with the genotypes *CSR1/CSR1-7* and *CSR1/CSR1-8* were phenotypically indistinguishable from wild-type plants in germination and vegetative growth (data not shown). The frequencies of seed abortion (Fig. 1b, c) were elevated to about 9% for *CSR1/CSR1-7* and about 19% for *CSR1/CSR1-8* (Table 2). Morphologically, two kinds of seed abortion could be distinguished. The most prevalent type was characterized by the failure of both the embryonic and integumentary tissues to develop. This was termed type II (Fig. 1b, c). A less severe type of seed abortion was also observed and characterized by the development of the integumentary tissues but not the embryonic tissues (Fig. 1b, c). These occurred at a lower frequency and were termed type I (Table 2). Both types could be observed in the same siliques of the heterozygous mutants (Fig. 1b, c). In wild-type siliques (Fig. 1a) seed abortion was occasionally observed but it was always the less severe type I seed abortion. This finding could explain the unexpected segregation ratios that were observed in Table 1.

Complementation of a *CSR1* loss-of-function mutant by a *CSR1-2* transgene

Transgenic lines carrying the *35S/CSR1-2/NOS* gene (Online Resource 1) were generated by transformation of Arabidopsis *csr1-7* hemizygous plants (*CSR1/CSR1-7*). This strategy was necessary as homozygous plants (*CSR1-7/CSR1-7*) were lethal. Ten lines that carried the *35S/CSR1-2/NOS* transgene and that segregated with the homozygous *CSR1-7/CSR1-7*

Table 1 Segregation of the *csr 1-7* and *csr 1-8* alleles

Genotype	Observed ratios			Expected ratios		
	n	*CSR1/CSR1*	*CSR1/CSR1-7/8*	*CSR1/CSR1* (%)	*CSR1/CSR1-7/8* (%)	*CSR1-7/8/CSR1-7/8* (%)
CSR1/CSR1-7	1,006	783 (78%)	223 (22%)	33	67	0
CSR1/CSR1-8	427	280 (66%)	180 (42%)	33	67	0

Seeds from selfed heterozygous plants were germinated on MS agar media with 25 mg/L glufosinate ammonium for *csr1-7* and 75 mg/mL sulfadiazine for *csr1-8* and genotyped by PCR. Sensitive plants were *CSR1/CSR1* and resistant plants *CSR1/CSR1-7* or *CSR1/CSR1-8*

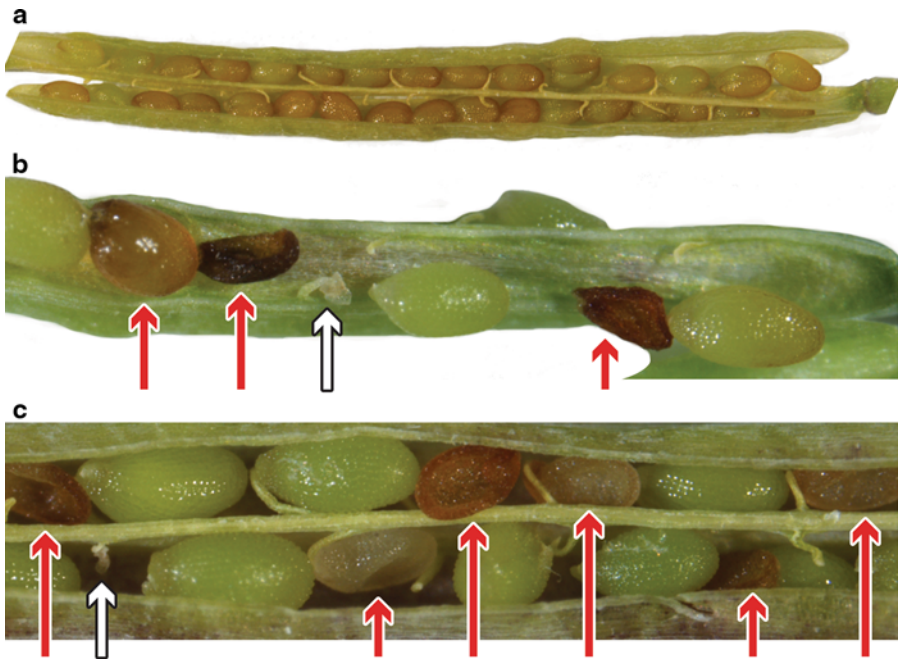

Fig. 1 Seed abortion phenotypes observed in *csr1-7* and *csr1-8* insertional mutants. Seed abortion phenotypes for type I are indicated by the *red arrows* and type II by *white arrows*. **a** Silique of wild-type Arabidopsis, ecotype Col-0. **b** Silique of *csr1-7*. **c** Silique of *csr1-8*

Table 2 Frequency of aborted seeds in heterozygous mutant lines of *csr1-7* and *csr1-8* and transgenic lines expressing *CSR1-2* in a homozygous *CSR1-7* background

Genotype	Frequency of aborted seeds[a] (mean percent ± SD)			Total seeds	Total plants
	Type I	Type II	Total		
CSR1/CSR1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	202	5
CSR1/CSR1-7	1.5 ± 0.4	7.6 ± 1.4	9.3 ± 1.4	931	25
CSR1/CSR1-8	6.4 ± 1.8	12.1 ± 3.1	18.5 ± 4.1	1,088	25
CSR1-2; CSR1-7/CSR1-7	24.2 ± 23.5	0.9 ± 1.3	25.1 ± 23.3	2,527	10[b]

[a] The type I and type II seed abortion phenotypes are illustrated in Fig. 1

[b] The 10 lines were randomly-selected independent transgenic events

background were recovered by screening with PCR primers shown in Online Resource 1. None of these lines carried a wild-type *CSR1* allele that could be vulnerable to gene silencing induced by transgenesis. Because the resident *CSR1-7* allele is already silenced by the insertion mutation the recovery of viable plants would have to be due to complementation by an active *CSR1-2* transgene (Online Resource 1). Any silencing of the *CSR1-2* transgene that might occur would be under negative selection pressure in this experiment and would not be recovered.

Substitution of *CSR1* with *CSR1-2* by transgenesis did not generate obvious differences in germination, growth and development of seedlings (Fig. 2a; data not shown). Interestingly, the seed abortion phenotype was highly variable among the 10 transgenic lines particularly for type I seed abortions (Table 2). Among the transgenic lines type I seed abortions ranged from 2.5 to 68%. The type II seed abortions were dramatically reduced or eliminated in the transgenic lines and ranged from 0 to 4.7%. Individual transgenic lines, such as line T1 (Fig. 2a), were found that were similar to wild-type plants in both vegetative and reproductive growth (i.e. 2.5% type I and 0% type II seed abortion) revealing that complementation of the loss-of-function mutation could be achieved by

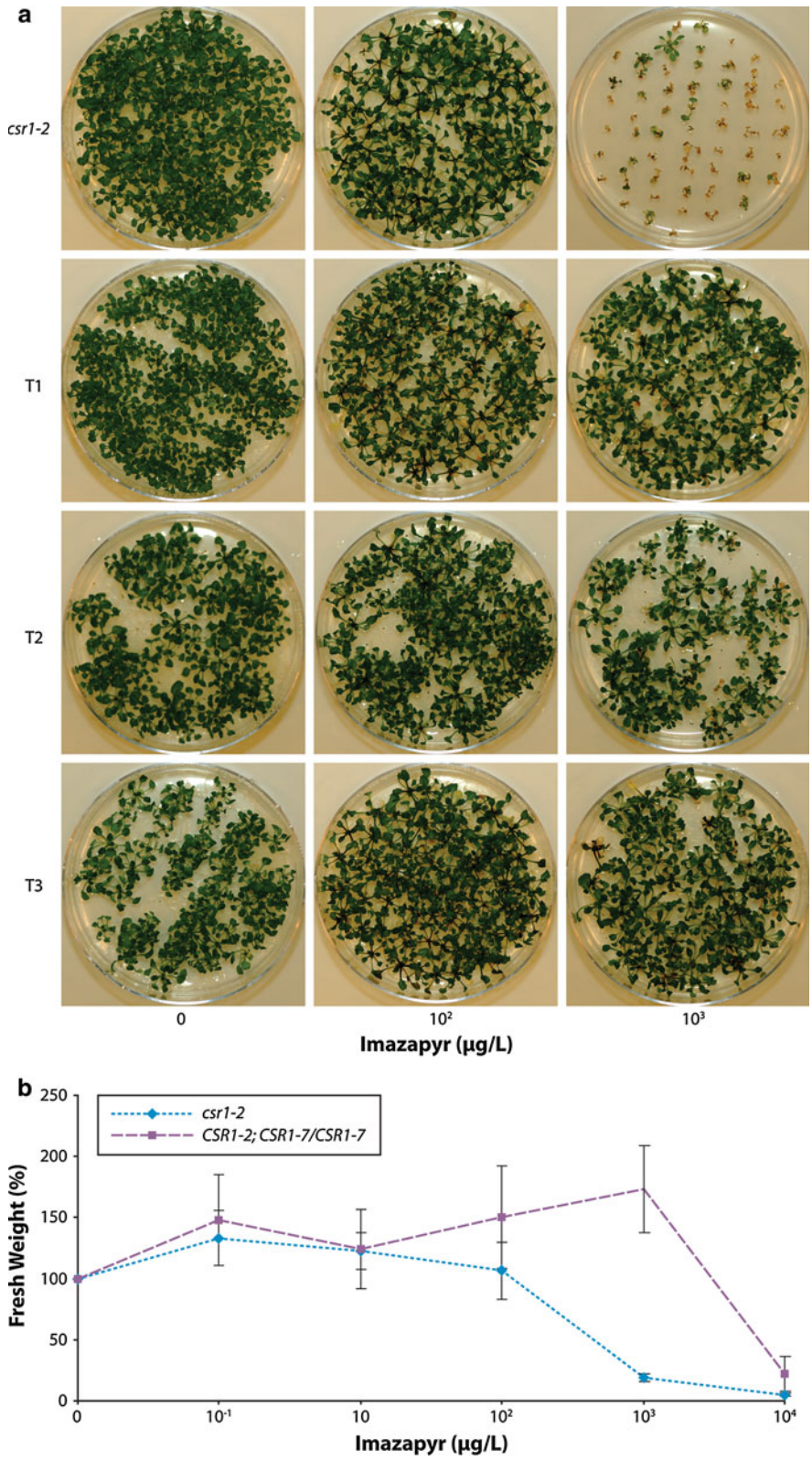

Fig. 2 Growth of the *csr1-2* mutant and transgenic lines expressing *CSR1-2* in a homozygous *CSR1-7* background in the presence of imazapyr. **a** The germination and growth of seedlings after 14 days for the *csr1-2* mutant and transgenic lines T1, T2, and T3 in the presence of 0, 10^2 and 10^3 µg/L imazapyr. **b** Increases in fresh weights were measured after 14 days of growth on media with varying concentrations of imazapyr. The fresh weights are expressed as a percentage of the fresh weight on growth without imazapyr. The values for the transgenic lines were calculated as the average ± SE for three independent transgenic lines, T1, T2, and T3, shown in Fig. 1a

insertion of the *35S/CSR1-2/NOS* gene without the introduction of significant pleiotropic effects on growth and development. This also suggested that the seed abortion phenotype in the heterozygous (*CSR1/CSR1-7*) mutant line described earlier resulted directly from the *csr1-7* mutation (Fig. 1) and not from pleiotropic effects on other genes.

Enhanced herbicide-resistance conferred by transgenes

No differences in vegetative growth between the *csr1-2* substitution mutant and three randomly-selected transgenic lines carrying the *35S/CSR1-2/NOS* transgene, including T1, were observed (Fig. 2a); however, differences were discovered when they were grown in the presence of imazapyr. All three transgenic lines were about an order of magnitude more resistant to imazapyr than the *csr1-2* substitution mutant (Fig. 2a, b). Furthermore, the enhanced resistance was accompanied by more vigorous vegetative growth in the presence of imazapyr (Fig. 2b). The increases in fresh weight accumulation were associated with an average increase in dry matter accumulation of 14.9 ± 6.2% measured at 14 days of growth.

Pleiotropic effects on the transcriptome

Microarray analysis was performed on 14 day-old seedlings grown in the presence of 100 μg/L imazapyr. Of the approximately 24,000 genes represented on the Affymetrix ATH1 GeneChip microarray, 68, 159, and 52 were found to be up- or down-regulated by greater than twofold ($P < 0.05$) in lines T1, T2, and T3, respectively (Online Resource 2). Of these, only five transcripts were commonly differentially expressed in all three transgenic lines (Table 3). The most altered corresponded to the *CSR1-2* transgene. It was elevated by approximately 7.9-, 7.1-, and 9.7-fold in T1, T2 and T3, respectively, relative to the levels in the *csr1-2* mutant. The data indicated that the elevated *CSR1-2* transcript expressed from the 35S regulatory sequences was likely responsible for the elevated resistance to imazapyr and the elevated biomass accumulation in the presence of imazapyr. The other genes included a putative peroxidase and an expressed protein, which were upregulated and the iron-responsive transporter IRT1 and a putative 2-oxoglutarate-dependent dioxygenase, which were downregulated.

Discussion

AHAS is a critical and essential enzyme in the branched-chain amino acids (BCAA: valine, leucine and isoleucine) biosynthetic pathway. It catalyzes the first common step in the pathway and it is the target for end-product inhibition. The catalytic subunit, encoded by homologues of the Arabidopsis *CSR1* gene, carries substitution mutations for resistance to the AHAS-inhibitor group of systemic herbicides in all plants examined (Duggleby et al. 2008). Plant death resulting from inhibition of AHAS by herbicides or by *CSR1* silencing of the AHAS-encoding genes appears to occur through amino acid starvation (Höfgen et al. 1995). Herbicides such as the sulfonylureas and imidazolinones appear to bind to specific overlapping sites in the substrate-access channel of the catalytic subunit thus preventing access to the active site. In the *csr1-2* substitution mutant (Ser-653-Asn) AHAS

Table 3 Genes up- or down-regulated greater than twofold ($P < 0.05$) in all three transgenic lines expressing *CSR1-2* in a homozygous *CSR1-7* background as compared to the *csr1-2* mutant

Probe set ID	AGI	Annotation	T1		T2		T3	
			M[a]	P	M	P	M	P
252325_at	At3g48560	Acetolactate synthase	2.97	4.38×10^{-06}	2.84	4.38×10^{-06}	3.28	4.38×10^{-06}
256569_at	At3g19550	Expressed protein	1.03	4.87×10^{-04}	1.34	3.68×10^{-04}	1.60	1.75×10^{-05}
247326_at	At5g64110	Peroxidase, putative	1.63	6.14×10^{-05}	1.19	7.85×10^{-04}	1.55	6.14×10^{-05}
260150_at	At1g52820	2-oxoglutarate-dependent dioxygenase, putative	−1.16	3.86×10^{-04}	−2.43	2.19×10^{-05}	−1.84	7.45×10^{-05}
254550_at	At4g19690	Iron-responsive transporter (IRT1)	−1.26	7.39×10^{-03}	−2.24	8.77×10^{-05}	−1.08	3.24×10^{-04}

[a] M is log2 fold change and P is FDR-adjusted *P* value for Student's *t*-test

cannot bind imidazolinone herbicides, such as imazapyr, but the active site is unaltered (McCourt et al. 2006). There appear to be no other enzymatic targets of significance in plants as the presence of imidazolinones in *csr1-2* mutant plants does not induce physiological alterations or changes in transcription profiles that can be detected by microarray (Manabe et al. 2007). These findings indicate that the mutant enzyme activity is unaffected by the mutation and that other plant processes are not affected by imidazolinones; therefore, the mutant plants are equivalent to wild-type plants in all other aspects.

Although tissue-specific variants of AHAS are found in some species, such as *Brassica napus* (Rutledge et al. 1991), the essential housekeeping genes are expressed constitutively throughout the plant (Ouellet et al. 1992). The lethal phenotype of the Arabidopsis deletion mutants *csr1-7* and *csr1-8* confirm that AHAS encoded by *CSR1* is an essential enzyme. The recovery of 35S/*CSR1-2/NOS* transgenic lines that are homozygous for *CSR1-7* indicate that the constitutively-expressed transgene can fully complement the null mutant in the transgenic state.

In heterozygous *csr1-7* or *csr1-8* plants we found that a single wild-type allele of *CSR1* was sufficient for normal seed germination and vegetative plant development but defects in development appeared during the reproductive phase, specifically in seed development. Recent studies have shown that Arabidopsis embryos require an exogenous supply of amino acids for growth and development (Sanders et al. 2009). There is no evidence for transcriptional feedback regulation of AHAS levels in plants (Ouellet et al. 1994) and it is known that the *CSR1* promoter is a weak promoter in transgenic plants (Charest et al. 1990). In other species, such as *B. napus*, the levels of transcripts of the housekeeping genes homologous to *CSR1* are also low in the reproductive organs but not substantially lower than in the vegetative tissues (Ouellet et al. 1992). It is therefore likely that AHAS levels are not adequately regulated at the transcriptional or post-transcriptional levels to compensate for the reduction in gene dosage in the heterozygous *csr1-7* and *csr1-8* plants. It might be expected that BCAA supply to these organs was insufficient under conditions of reduced gene dosage through reduced levels of gene expression and thus AHAS activity. The use of the 35S promoter to elevate the level of *CSR1-2* transcript by sevenfold to tenfold appeared to overcome the limitation and restored seed production levels to those of wild-type plants in some transgenic but not all lines. The embryo abortion phenotype, in particular type I, was highly variable in frequency among the transgenic lines. The transgenic lines analyzed were homozygous for the *csr1-7* T-DNA insertion, so that expression controlled by the native promoter was eliminated and *CSR1-2* expression is completely dependent on the 35S promoter, which is known to be weak during early embryogenesis in some species (Sunilkumar et al. 2002). Variable expression of the inserted *CSR1-2* gene in the different transgenic lines during this developmental stage likely accounts for the observed variability in the seed abortion phenotype.

The introduction of *CSR1-2* transcripts in transgenic plants using the 35S regulatory sequences was accompanied by a small number of changes in the transcriptome. In addition to *CSR1-2*, four genes were differentially expressed in all three transgenic lines. These small changes in gene transcription may be related to the higher levels of expression of the *CSR1-2* gene in the transgenic plants, although the genes do not have any known connection with AHAS or amino acid biosynthesis. Alternatively, they may be the result of the more vigorous vegetative growth observed in the presence of imazapyr.

A search for changes in stress-related genes induced by imazapyr (Das et al. 2010) failed to indicate that any of the plants were under physiological stress. The transgenic plants were therefore very similar transcriptionally to the mutant *csr1-2* plants. They had the added advantage of more robust growth in the presence of herbicide and a greater level of herbicide resistance.

In summary, this study provides insight into the differences and similarities of mutants and transgenic plants with the same novel trait, herbicide resistance, at the physiological and transcriptional levels. Furthermore it illustrates some of the advantages of transgenesis over mutagenesis by allowing for the manipulation of multiple regulatory domains that can affect gene expression and enzyme properties simultaneously. The data extends previous studies (El Ouakfaoui and Miki 2005; Manabe et al. 2007) by showing that transgenesis may be employed to generate plants that are similar transcriptionally to both wild-type and mutant plants, except for the gene encoding the novel trait. This kind of data is needed to

develop scientifically sound regulatory policies for the integration of new crops to meet the unique challenges of future agriculture.

Acknowledgments We thank the Arabidopsis Biological Resource Center for providing seeds of *csr1-2*^{D}, *csr1-7* and *csr1-8* and American Cyanamid (currently BASF) for providing the imazapyr, respectively. The research was funded by the Plant Biosafety Office, Plant Products Directorate, Canadian Food Inspection Agency and Feeds Section, Animal Health and Production Division, Animal Products Directorate, Canadian Food Inspection Agency. The funders did not influence the research, data, conclusions or publication.

Conflict of interest The authors have declared that no conflict of interests exist.

References

Abdeen A, Schnell J, Miki B (2010) Transcriptome analysis reveals absence of unintended effects in drought-tolerant transgenic plants overexpressing the transcription factor *ABF3*. BMC Genomics 11:69

Batista R, Saibo N, Lourenço T, Oliveira MM (2008) Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. Proc Natl Acad Sci USA 105:3640–3645

Baudo MM, Lyons R, Powers S, Pastori GM, Edwards KJ, Holdsworth MJ, Shewry PR (2006) Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. Plant Biotechnol J 4:369–380

Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH, Gatehouse AMR, Kärenlampi S, Kok EJ, Leguay J–J, Lehesranta S, Noteborn HPJM, Pedersen J, Smith M (2004) Unintended effects and their detection in genetically modified crops. Food Chem Toxicol 42:1089–1125

Charest PJ, Hattori J, DeMoor J, Iyer VN, Miki BL (1990) In vitro study of transgenic tobacco expressing *Arabidopsis* wild type and mutant acetohydroxyacid synthase genes. Plant Cell Rep 8:643–646

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Das M, Reichman JR, Haberer G, Welzl G, Aceituno FF, Mader MT, Watrud LS, Pfleeger TG, Gutiérrez RA, Schäffner AR, Olszyk DM (2010) A composite transcriptional signature differentiates responses towards closely related herbicides in *Arabidopsis thaliana* and *Brassica napus*. Plant Mol Biol 72:545–556

Davies H (2010) A role for "omics" technologies in food safety assessment. Food Control 21:1601–1610

Duggleby RG, McCourt JA, Guddat LW (2008) Structure and mechanism of inhibition of plant acetohydroxyacid synthase. Plant Physiol Biochem 46:309–324

El Ouakfaoui S, Miki B (2005) The stability of the Arabidopsis transcriptome in transgenic plants expressing the marker genes *nptII* and *uidA*. Plant J 41:791–800

Höfgen R, Laber B, Schüttke I, Klonus A-K, Streber W, Pohlenz H-D (1995) Repression of acetolactate synthase activity through antisense inhibition: molecular and biochemical analysis of transgenic potato (*Solanum tuberosum* L. cv Désirée) plants. Plant Physiol 107:469–477

Kogel K-H, Voll LM, Schäfer P, Jansen C, Wu Y, Langen G, Imani J, Hofmann J, Schmiedl A, Sonnewald S, von Wettstein D, Cook RJ, Sonnewald U (2010) Transcriptome and metabolome profiling of field-grown transgenic barley lack induced differences but show cultivar-specific variances. Proc Natl Acad Sci USA 107:6198–6203

Kuiper HA, Kok EJ, Engel K-H (2003) Exploitation of molecular profiling techniques for GM food safety assessment. Current Opin Biotechnol 14:238–243

Lehesranta SJ, Davies HV, Shepherd LVT, Nunan N, McNicol JW, Auriola S, Koistinen KM, Suomalainen S, Kokko HI, Kärenlampi SO (2005) Comparison of tuber proteomes of potato varieties, landraces, and genetically modified lines. Plant Physiol 138:1690–1699

Manabe Y, Tinker N, Colville A, Miki B (2007) CSR1, the sole target of imidazolinone herbicide in *Arabidopsis thaliana*. Plant Cell Physiol 48:1340–1358

McCourt JA, Duggleby RG (2006) Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. Amino Acids 31:173–210

McCourt JA, Pang SS, King-Scott J, Guddat LW, Duggleby RG (2006) Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. Proc Natl Acad Sci USA 103:569–573

McHughen A (2007) Fatal flaws in agbiotech regulatory policies. Nature Biotechnol 25:725–727

McHughen A, Smyth S (2008) US regulatory system for genetically modified [genetically modified organism (GMO), rDNA or transgenic] crop cultivars. Plant Biotechnol J 6:2–12

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

Ouellet T, Mourad G, Brown D, King J, Miki B (1994) Regulation of acetohydroxyacid synthase activity levels in transgenic tobacco. Plant Sci 102:91–97

Park JR, McFarlane I, Phipps RH, Ceddia G (2011) The role of transgenic crops in sustainable development. Plant Biotechnol J 9:2–21

Parrott W, Chassy B, Ligon J, Meyer L, Petrick L, Zhou J, Herman R, Delaney B, Levine M (2010) Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. Food Chem Toxicol 48:1773–1790

Parry MAJ, Madjwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, Labhilili M, Phillips AL (2009) Mutation discovery for crop improvement. J Ext Bot 60:2817–2825

Rosso MG, Li Y, Strizhov N, Reiss B, Dekker K, Weisshaar B (2003) An *Arabidopsis thaliana* T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. Plant Mol Biol 53:247–259

Rutledge RG, Ouellet T, Hattori J, Miki BL (1991) Molecular characterization and genetic origin of the *Brassica napus*

acetohydroxyacid synthase multigene family. Mol Gen Genet 229:31–40

Sanders A, Collier R, Trethewy A, Gould G, Sieker R, Tegeder M (2009) AAP1 regulates import of amino acids into developing Arabidopsis embryos. Plant J 59:540–552

Sessions A, Burke E, Presting G, Aux G, McElver J, Patton D et al (2002) A high-throughput Arabidopsis reverse genetics system. Plant Cell 14:2985–2994

Smyth S, McHughen A (2008) Regulating innovative crop technologies in Canada: the case of regulating genetically modified crops. Plant Biotechnol J 6:213–225

Sunilkumar G, Mohr L, Lopata-Finch E, Emani C, Rathore KS (2002) Developmental and tissue-specific expression of CaMV 35S promoter in cotton as revealed by GFP. Plant Mol Biol 50:463–479

Tan S, Evans RR, Dahmer ML, Singh BK, Shaner DL (2005) Imidazolinone-tolerant crops: history, current status and future. Pest Manag Sci 61:246–257

Waugh R, Leader DJ, McCallum N, Caldwell D (2006) Harvesting the potential of induced biological diversity. Trends Plant Sci 11:71–79