

Full Length Research Paper

The suppression of *Arabidopsis* succinic semialdehyde dehydrogenase (SSADH) phenotype by using ethyl methane-sulfonate mutagenesis (EMS)

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The disruption of the succinic semialdehyde dehydrogenase (SSADH) function in *Arabidopsis* leads to aberrant growth phenotype. In human, the SSADH malfunction leads to a condition known as γ -hydroxybutyrate (GHB) aciduria. It is characterized by the accumulation of GHB as well as abnormal growth. GHB aciduria is treated pharmacologically with compounds analogous to gamma-aminobutyrate (GABA). One of these compounds called vagabatin effectively suppressed the *ssadh* phenotype in *Arabidopsis*. Furthermore, the *Arabidopsis ssadh* phenotype has been suppressed genetically by simultaneous mutation in the *POP2* gene. In the present study, the presence of alternative solutions for suppression of the *ssadh* phenotype was investigated. For that, four ethyl methanesulfonate treated *ssadh* lines namely 7D, 13J, 17J and 21H were characterized phenotypically and chemotypically. Three-week-old plants of the suppressor lines grew much better than the parent *ssadh* line and resembled the wild type. The suppressor lines also accumulated reduced amounts of GABA and GHB. PCR based mapping using Sequence Characterized Amplified Region (SCAR) markers located the mutations to chromosome 2 and chromosome 5, suggesting the involvement of at least two mutations in suppression of the *ssadh* phenotype. Deep whole-genome sequencing of line 17J identified several mutations that induced amino acid change, frame shift and a stop codon. The findings showed that there are more possibilities to suppress the *ssadh* phenotype; furthermore, it provides an opportunity to identify genes that interact with the GABA shunt.

Key words: Gamma-aminobutyrate (GABA), shunt; *succinic semialdehyde dehydrogenase (SSADH)* mapping, *Arabidopsis*, succinic semialdehyde (SSA), γ -hydroxybutyrate (GHB).

INTRODUCTION

Succinic semialdehyde dehydrogenase (SSADH) is a Gamma-aminobutyrate (GABA)-shunt enzyme that catalyzes the oxidation of succinic semialdehyde (SSA) to succinate in the presence of NAD^+ . *Arabidopsis* is an

annual plant with short life cycle. In plant genetics study, *Arabidopsis* is often used as a model organism due to its small genome size and short seed to seed cycle. Its genome contains a single copy of SSADH encoding gene

(Busch and Fromm, 1999). Knockout of this single gene leads to an accumulation of reactive oxygen species (ROS), a stunted growth (dwarfism), a necrosis on leaves and hypersensitivity to environmental stresses (Fait et al., 2004; Bouche et al., 2003). Furthermore, this abnormal growth phenotype was associated with an accumulation of gamma hydroxybutyric acid (GHB) and hydrogen peroxide in tissues (Fait et al., 2004). In mammals, deficiency in SSADH activity causes a disorder called GHB aciduria, which is manifested by a high level of GHB in the physiological fluids (Jakobs et al., 1981; Hogema et al., 2001).

GHB aciduria in mammals is treated with several drugs. The most commonly used one is γ -vinyl- γ -aminobutyrate (Vagabatratin), a GABA analogue which binds to GABA transaminase irreversibly and prevents the degradation of GABA (Knerr et al., 2007). A similar rescue of the *ssadh* phenotype has been reported in *Arabidopsis*. *Arabidopsis ssadh* mutant treated with vagabatratin showed a reduced accumulation of ROS, reduced cell death and an improved growth compared to the *ssadh* mutant (Fait et al., 2004). In 2008, Ludewig et al. (2008) reported a complete suppression of the *ssadh* phenotype by simultaneous knockout of the *POP-2* gene, whose encoded protein functions upstream of the SSADH. This observation suggested that blocking the degradation of GABA is sufficient to suppress the *ssadh* phenotype.

Functional characterizations of genes are usually done using either forward or reverse genetics approaches. With the reverse genetics approach, one way of mutating genes is by insertion of T-DNA. However, this approach is random and lack specificity. Recent advancements in biotechnological tools allowed targeted edition of genes. CRISPR-Cas technology is one of those technologies that allow targeted modifications of genes (Eid et al., 2016; Schimel et al., 2014). Hyun et al. (2014) used CRISPR-Cas technology to mutagenize FLOWERING LOCUS T and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE genes. Site directed mutagenesis is another method that allows modification of specific amino acids thereby altering the functional properties of proteins. Majeed et al. (2015) and Fontenot et al. (2015) employed site directed mutagenesis to alter single amino acid in UGT76E1 and PhT1 proteins, respectively. The substitution of amino acid threonine (T) by alanine (A) at the position 134 of the UGT76E1 protein abolished the glycosyltransferase activity of the protein (Majeed et al., 2015). Furthermore, the replacement of tyrosine (Y) by Aspartic acid (D) at the 312 position of the PhT1 protein enhanced its Pi transport activity (Fontenot et al., 2015).

Forward genetics approach, on the other hand, involves the induction of random mutation, assessment of

phenotypic changes and identification of the mutations responsible for phenotypic change. Several mutagens such as chemicals and rays are used to induce random mutation in the genome of an organism. Ethyl methanesulfonate (EMS) is one of those chemicals which are used as a mutagen in biological studies. EMS induces single nucleotide polymorphisms (SNPs) usually by substitution of bases (Kim et al., 2006). Therefore, EMS mutagenesis has been used widely in forward genetics to identify functions of genes. Previously, Ludewig et al. (2008) isolated two point mutations within the *pop2* ORF that suppressed the *ssadh* phenotype. In the same work, the authors reported the isolation of several EMS suppressor lines. Despite the improved phenotype of the EMS lines, the mode of suppression and genes involved in the process are unknown.

Here, it was hypothesized that mutations in genes other than the *POP2* are responsible for suppression of the *ssadh* phenotype. First, the phenotype and chemotype of four EMS suppressor lines was characterized and named hereafter as 7D, 13J, 17J and 21H. The growth of all four EMS suppressor lines is much better than the parent *ssadh* plant. The point mutations that suppressed the *ssadh* phenotype of line 17J were mapped to chromosome 2 and chromosome 5, confirming that mutations in genes other than in *POP2* were responsible for suppression of the *ssadh* phenotype. Deep sequencing of the entire genome of line 17J identified several candidate SNPs that led to amino acid substitution, frame shift and a premature stop codon. Our findings provide a basis for further studies to identify new GABA-shunt interacting genes.

METHODOLOGY

Generation of EMS-suppressor lines

The generation of the EMS suppressor lines was reported previously (Ludewig et al., 2008). Briefly, seeds of *ssadh-2* mutant were incubated in water containing 0.23% ethyl methanesulfonate (EMS) for 12 h at room temperature. Then, the seeds were washed several times with water and finally dried. The suppressor lines were isolated by visual observation after sowing the seeds on soil.

Phenotypic analysis

The phenotype lines 7D, 13J, 17J and 21H was compared to wild type and *ssadh* homozygous plants. For that, seeds of EMS suppressor lines were germinated and grown on soil. After four weeks of growth pictures were taken. Furthermore, the growth of EMS suppressor lines was analyzed on agar plate containing 0.3 mM SSA. For that, seeds of Wt, 7D, 13J, 17J, 21H and *pop2* x *ssadh* mutant (*gs*) were germinated and grown on ½ MS plate without and with 0.3 mM SSA. Two weeks after germination the

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fresh shoot weight and root length of plantlets was determined.

Metabolite analysis

The chemotype of lines 7D, 13J, 17J and 21H was compared with the wild type and the *ssadh* mutant. For that, leaf material (100-200 mg) was collected from four-week-old wild type, *gad1/2-ssadh* and EMS suppressor lines, and was snap-frozen in liquid nitrogen. To get sufficient material from *ssadh* mutant, the entire shoots of six-week-old plants excluding flowers were used. Metabolite extraction and measurement was carried out as described previously (Renault et al., 2010; Mekonnen et al., 2016).

Isolation of mapping population

First, line 17J was crossed to *Landsberg erecta* (*Ler*) wild type. F1 seeds were sown on soil and the resulting F1 plants were selfed. Next, F2 plants were screened for homozygosity of the T-DNA insertion in the *SSADH* gene by PCR and also for an improved growth visually. Those lines with homozygous *ssadh* mutation and improved growth were selected as mapping population. However, this procedure was very laborious, since only few plants tested belonged to the mapping population. Hence, preliminary selection was adopted on agar plates. For that, sterilized F2 seeds were germinated and grown on ½MS plates. A week after germination, only yellowish plantlets were transferred to fresh ½MS plates and after another week of growth the plantlets were transferred to soil. Approximately two weeks after transferring to soil, plants belonging to the mapping population were screened by PCR and visual observation.

Mapping of EMS-induced mutations

To determine the position of SNPs that suppressed the *ssadh* phenotype, genomic DNA was extracted from the mapping populations as described previously (Scholz et al., 2015). Markers discriminating between the Columbia (*Col-0*) and *Landsberg erecta* (*Ler*) fragments were designed on all five chromosomes (Figure S1 and Table S1). PCR was performed on genomic DNA extracts of the mapping population. For genomic DNA extraction the procedure described by Scholz et al. (2016) was used. The abundance of *Col-0* and *Ler* fragments was counted as follows. A single band per lane corresponding to *Col-0* or *Ler* is counted as two and double bands per lane counted one for each genotype. The number of bands was summed up and the percent of *Col* fragment was calculated as shown by the following formula:

$$\% Col = \text{no. of } Col - 0 \text{ fragments} \div \text{total number of fragments} \times 100$$

Genomic DNA extraction for whole genome sequencing

High quality genomic DNA was extracted from three-week-old 17J line germinated and grown on soil. For that ~100 mg of leaf material was collected from line 17J and immediately frozen in liquid nitrogen. The extraction of the genomic DNA was carried out according to the DNeasy Plant Mini Kit protocol (Cat. No. 69104). The concentration of the DNA was determined using a Nano Drop.

RESULTS AND DISCUSSION

The EMS suppressor lines grow better than the *ssadh* mutant

The growth of *Arabidopsis ssadh* mutants is severely

inhibited (Ludewig et al., 2008; Fait et al., 2004). However, the simultaneous knockout of the *POP2* gene completely suppressed the *ssadh* phenotype (Ludwig et al., 2008). In the present study, the *ssadh* phenotype was suppressed by random mutations induced by EMS. The growth of EMS suppressor lines (7D, 13J, 17J and 21H) was much better than the *ssadh* mutant (Figure 1A). Three-week-old plants of line 17J and 21H resemble the wild type and *pop2 x ssadh* mutant, confirming the suppression of the *ssadh* phenotype (Figure 1A). To ensure that the T-DNA insertion in the *SSADH* gene was stable, the EMS suppressor lines were genotyped using gene- and T-DNA-specific primer combinations. Indeed, all lines were homozygous for T-DNA insertion; since, the PCR using gene specific forward and reverse primers did not yield a product (Figure 1B).

Furthermore, if EMS suppressor lines would be sensitive to SSA treatments differently compared to the controls was tested. Similar to soil grown plants, there was no difference in shoot growth between the EMS suppressor lines and the controls (*Wt* and *gs*) on ½ MS plates (Figure 2A). However, a shorter root was measured in all EMS lines when compared to the wild type on ½ MS plates (Figure 2B). This might be due to the accumulation of GABA after the disruption of the *SSADH* function. The accumulation of GABA in *SSADH* deficient *Arabidopsis* plants has previously been reported (Ludwig et al., 2008; Fait et al., 2004). A high GABA level is associated with reduced root growth. Ramesh et al. (2015) showed a reduced growth of wheat roots after 10 mM exogenous GABA application. Treatment of 0.3 mM SSA reduced the growth of all lines including the wild type (Figure 2C), an observation in line with the previous report (Mekonnen and Ludwig, 2016). The suppression of the *ssadh* phenotype by EMS induced mutation could be in two ways; first by preventing the production of the toxic intermediates; second, by promoting the degradation of the toxic intermediate(s). The phenotype of EMS lines following SSA treatment probably suggests the first mode of suppression of the *ssadh* phenotype. The phenotypic difference among EMS lines might indicate the presence of alternative ways to rescue the *ssadh* phenotype.

EMS induced mutations reduced the GABA and GHB levels

Some of the features of the *ssadh* chemotype include the accumulation of high GABA and GHB in tissues (Ludwig et al., 2008). In the present report, the abundance of GHB and its correlation with the suppression an *ssadh* phenotype was investigated. Interestingly, the GHB content was reduced by more than 30-fold in shoots of EMS suppressor lines compared to the *ssadh* mutant (Figure 3B). Similarly, the GABA content in EMS suppressor lines was reduced by more than 50% compared to the *ssadh* mutant (Figure 3A). The reduction in levels of GABA and GHB suggests that prevention of

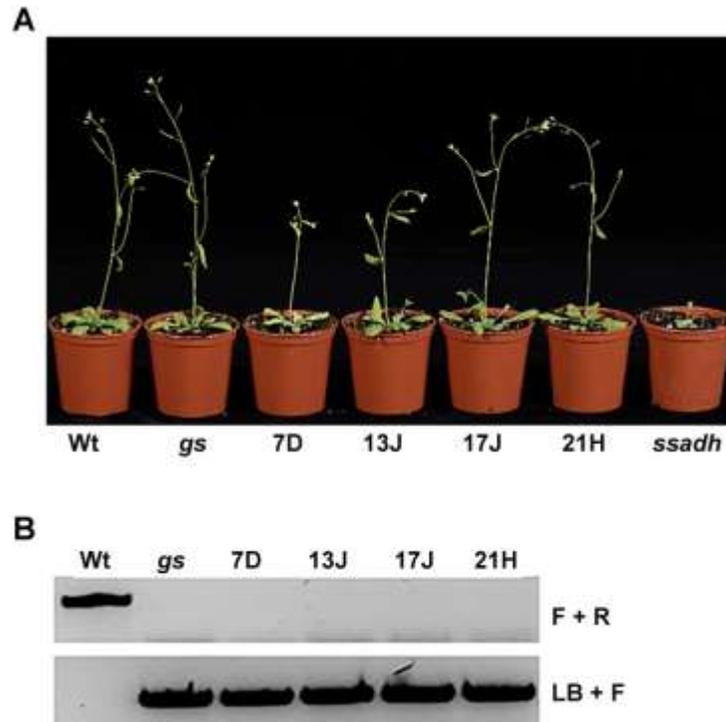


Figure 1. Phenotype of Wt, *pop2 x ssadh*, *ssadh* and four EMS lines (A), and genotyping of EMS lines (B); seeds of Wt, *pop2 x ssadh* and four EMS lines (7D, 13J, 17J and 21H) were germinated and grown on soil; pictures were taken from three-week-old plants; phenotype are representatives of at least 10 plants of similar phenotype; screening of EMS suppressor lines for T-DNA insertion by using gene specific primers (F + R) and a combination of gene specific and T-DNA specific primers (LB); *gs*-represents the *pop2 x ssadh* double mutant.

toxic intermediates production was probably the mode of *ssadh* phenotype suppression. Intriguingly, lines 7D and 13J exhibited a relatively reduced shoot growth phenotype compared to 17J and 21H (Figure 1A) despite accumulating several folds less GHB in the tissue, suggesting that the severity of the *ssadh* phenotype is not correlated to the GHB level. Akaboshi et al. (2003) reported the absence of correlation between GHB levels and clinical features in mammals. Our recent report also showed that GHB induced damage in *Arabidopsis* is modest and zero in yeast at high concentrations (Mekonnen and Ludewig et al., 2016). This observation adds further evidence to a previous report that GHB is not the main cause for *ssadh* phenotype.

SNPs in at least two genes suppressed the *ssadh* phenotype

The PCR based mapping system using Sequence Characterized Amplified Region (SCAR) markers has been used widely to locate SNP's. The presence of natural polymorphisms between two *Arabidopsis*

ecotypes (Col and *Ler*) lays a good platform for designing SCAR markers. For example, Konieczny and Ausubel (1993) identified an average of one nucleotide change in 261 base pairs between Col and *Ler* ecotypes that comprised recognition sites for CAPS marker. Based on the nucleotide polymorphisms between these two ecotypes, several SCAR markers were designed spanning the shorter and longer arms of all five chromosomes (Figure 4 and Table S1). Using a combination of PCR genotyping and visual analysis, more than 90 lines belonging to the mapping populations was isolated for line 17J. Interestingly, PCR analysis using markers 1.8, 2.6 and 5.9 identified three regions on chromosome 1, 2 and 5, respectively, which is rich in Col-0 fragments (Table 1A). The lower arm of chromosome 1 is a position where the *ssadh* T-DNA insertion was located. Therefore, it was not surprising to see dominance of Col-0 fragments. To confirm the position of the SNPs, additional PCRs were performed by using more lines from the mapping population and found that the mutations were, indeed, in chromosome 2 and 5 (Table 1B). Those findings show that the *ssadh* phenotype was suppressed by at least two mutations in two different

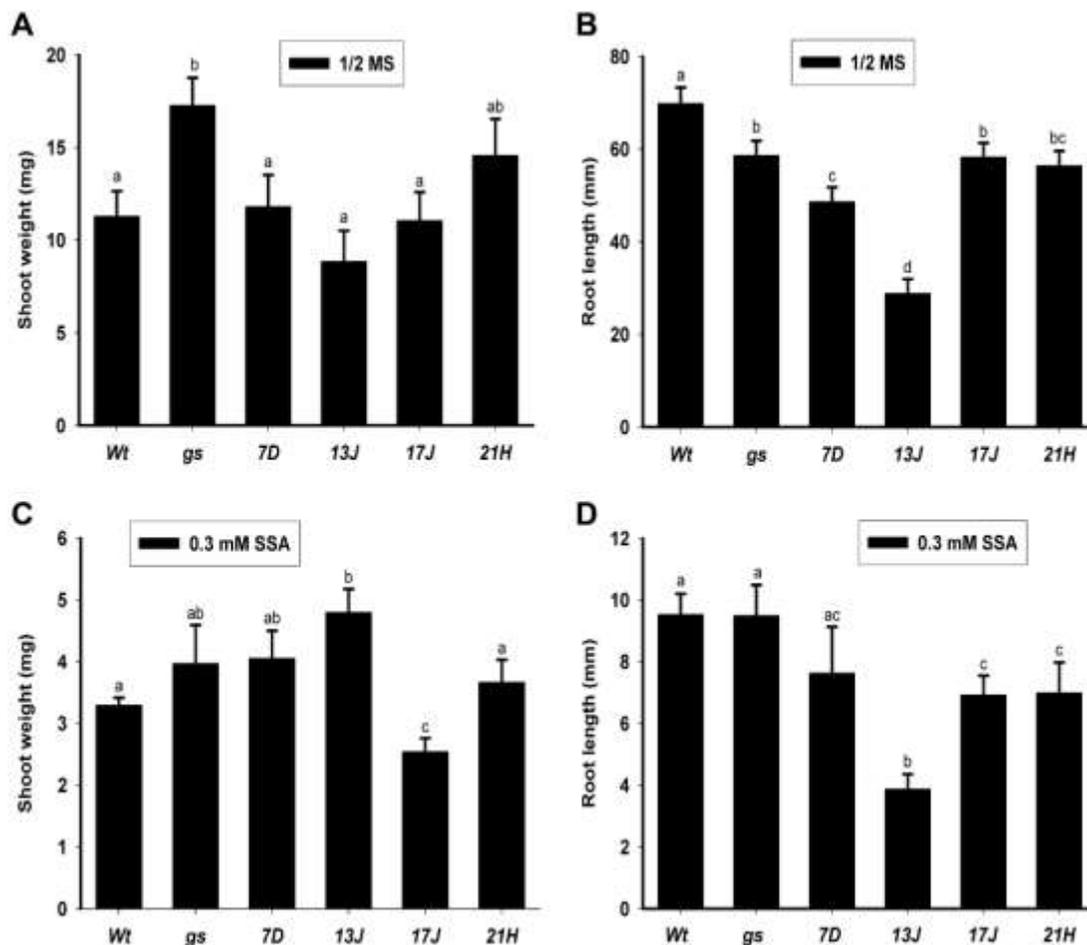


Figure 2. Phenotypic characterization of Wt, *pop2 x ssadh* and four EMS suppressor lines (7D, 13J, 17J and 21H); seeds of Wt and mutant lines were germinated and grown on 1/2 MS plate without (A&B) and with 0.3 mM SSA (C&D); shoot fresh weight (A&C) and root length (B&D) were measured after two weeks of growth; bars represent the standard error of means; different letters indicate statistical significances after students *t*-test; *gs*- represent the *pop2 x ssadh* mutants.

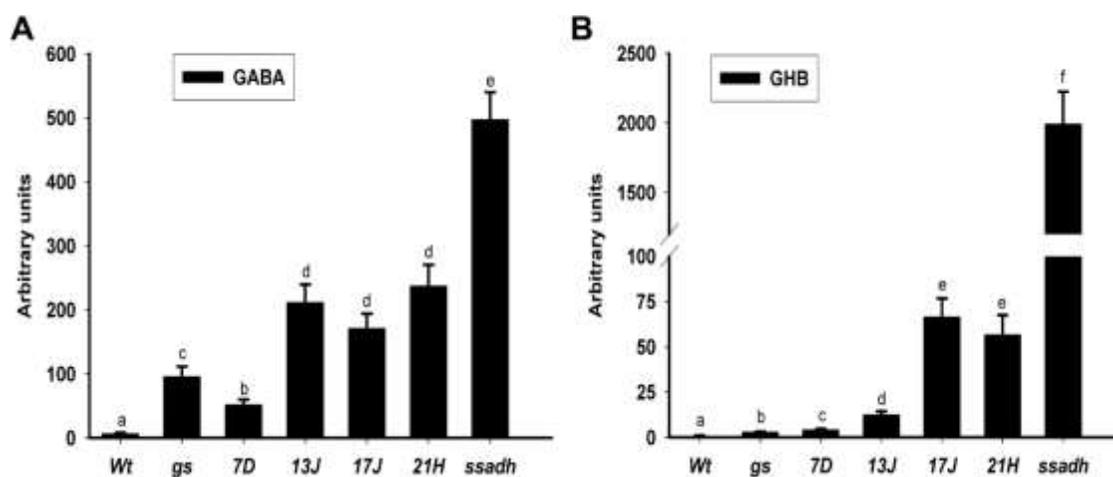


Figure 3. Analysis of GABA (A) and GHB (B) in four-week-old wild type, *pop2 x ssadh* (*gs*), four EMS lines (7D, 13J, 17J and 21H) and six-week-old *ssadh* plants values are means of at least five independent plants; bars represent the standard error of means; different letters indicate statistical significances after students *t*-test; *gs*- represent the *pop2 x ssadh* mutants.

Table 1. Preliminary (A) and detailed (B) analysis of EMS induced SNPs in line 17J; to determine the position of SNPs, primers that span all five chromosomes were used (A); to verify the positions additional plants and primers were used (B).

Chromosomes	Marker name	% Col	No. of independent lines
A. Rough SNP mapping			
1	1.1	38.8	20
	1.8	88.8	20
2	2.1	47.2	20
	2.6	94.1	20
	2.7	33.0	20
3	3.1	41.6	20
	3.7	52.7	20
4	4.2	22.2	20
	4.7	44.4	20
5	5.3	66.6	20
	5.9	100.0	20
B. Detailed SNP mapping			
2	2.6	95.2	72
5	5.8	89.5	72
	5.9	91.5	72
	VG	95.0	72

chromosomes. Despite the absence of a segregation analysis data, the proportion of plants belonging to the mapping population in the F2 generation was close to 1/16.

Deep sequencing of line 17J genome uncovered several candidate mutations

The introduction and accessibility of full genome sequencing facilities greatly eased the identification of SNP's. The whole genome sequencing of line 17J identified hundreds of point mutations compared to the reference allele. To determine the regions of interest on chromosome 2 and 5, the following calculations were made. Markers 2.6 and VG on chromosome 2 and 5, respectively, yielded a 95% Col-0 fragment and 5% *Ler* fragment (Table 1B). Centimorgan (cM) is a unit of measuring the linkage between genes on the same chromosome. The 5% *Ler* fragments in chromosomes 2 and 5 (Table 1B) indicate that five crossing over has occurred between the markers and EMS induced SNP's. From the above definition, it can be deduced that the markers and EMS induced SNPs are 5 cM apart. Although the conversion of 1 cM to base pair depends on the position on the chromosome, the average number was calculated to be 208950 and 203800 base pairs in chromosome 2 is 5, respectively (<http://www.arabidopsis.org/servlets/mapper>). The region of interest was obtained by multiplying the estimated

base pairs with 5. To ensure the selection of the right SNPs, a 5% cut off value was set for the ratio of reference calls to alternative calls, that is, any ratio value higher than 0.05 was discarded.

Sequence analysis revealed hundreds of SNPs within the desired region of both chromosomes. However, only 47 and 22 SNPs were true in chromosome 2 and 5, respectively, since more than 95% of the calls favored against the reference allele. The SNPs were located within the exon, intron, 3' UTR or 5' UTR of the gene. Among all SNPs, 14 and 10 point mutations in chromosome 2 and 5, respectively, were located within the exon of genes (Table 2). The point mutations induced amino acid changes, premature stop codon and a frame shift (Table 2). Furthermore, there were some notable changes in terms of the amino acid polarity and charge in both chromosomes. For example, the substitution of Gly by Asp, Arg by Glu, Gly by Arg, and Gly by Glu were the notable ones in genes of both chromosomes (Table 2). Such substitution could change the function of the protein. Single amino acid substitutions have previously been shown to alter the functional property of proteins. Lee et al. (2015) reported the enhanced peroxidase and chaperone activity of 2-Cys Peroxiredoxin (2-Cys Prx A) protein following the substitution of Cys150 for Ser150. Similarly, the replacement of Thr134 by Ala134 of Arabidopsis UGT76E1 protein abolished its glycosyltransferase activity (Majeed et al., 2015). Such functional alteration of proteins will likely have consequences on the phenotype (Ng and Henikoff,

Table 2. SNPs identified in exon of genes in chromosome 2 (A) and chromosome 5 (B) of line 17J.

S/N	Gene ID	Reference/Alternative alleles	Amino acid change	Annotation
A. Chromosome 2				
1	At2g25340	0.050	Ser to Asn	Vesicle associated membrane proteins
2	At2g25640	0.000	frame shift	Transcription elongation factor S-II protein
3	At2g25660	0.032	Arg to Glu	Embryo defective 2410
4	At2g25790	0.045	Pro to Leu	Protein serine/threonine kinase activity
5	At2g28930	0.033	Pro to Ser	Product protein kinase 1B
6	At2g29065	0.000	Leu to Phen	GRAS family transcription factor
7	At2g29660	0.026	Asp to Asp	ZINC finger (C2H2 type) family protein
8	At2g30300	0.023	Ser to Ser	Major facilitator superfamily protein
9	At2g30500	0.030	Ala to Threo	Kinase interacting-like proteins
10	At2g31080	0.000	frame shift	Non-LTR retrotransposone family
11	At2g31370	0.000	Pro to Ser	bZIP transcription factor family protein
12	At2g31560	0.050	Gln to stop	Protein of unknown function
13	At2g32310	0.016	Phe to Phe	CCT motif family protein
14	At2g32730	0.022	Leu to Phen	26S proteasome regulatory complex, non-ATPase sub complex, Rpn2/Psmd1 subunit
B. Chromosome 5				
1	At5g51340	0.00	Ileu to Ileu	Tetratricopeptide repeat like super family protein
2	At5g51630	0.04	Asp to Asp	Disease resistance protein family
3	At5g51660	0.04	Pro to Leu	Cleavage and polyadenylation specificity factor
4	At5g52115	0.00	Frame shift	CDS ribonuclease H superfamily
5	At5g52400	0.00	Pro to Ser	Member of CYP715A
6	At5g53150	0.03	Gly to Arg	DNAJ heat shock protein
7	At5g53660	0.05	Glu to Glu	Growth Regulating Factor 7
9	At5g53850	0.04	Gln to His	Dehydratase enolase phosphatase complex 1
10	At5g53970	0.01	Gly to Glu	Tyrosine aminotransferase 7

2006). The suppression of the *ssadh* phenotype was, indeed, the manifestation of the amino acid change. To our knowledge no report has been published indicating interaction between these candidate genes and the GABA shunt.

Conclusion

The only known way of suppressing of the *ssadh* phenotype in *Arabidopsis* is through mutations in *POP2* gene. The finding here provided potential candidate genes that have never been reported to interact with the GABA shunt before. The candidate genes can be used to study their role in suppression of *ssadh* phenotype as well as their mode of interaction with the GABA shunt. Considering the diverse roles of the GABA shunt in plant growth and development, there is a promise of identifying new enzymatic pathways. Furthermore, in humans the disorder resulting from *ssadh* malfunction is treated only with chemical drugs (GABA-analogues) that competitively inhibit the function of GABA-T (*POP2*). The identification of additional pathways that suppress the *ssadh* phenotype is helpful; since, the information could

be used as an input for the discovery of drugs in GHB aciduria treatment of mammals. Taken together, the result of the study lays a good basis to identify new genes and possibly pathways that interact with the GABA shunt.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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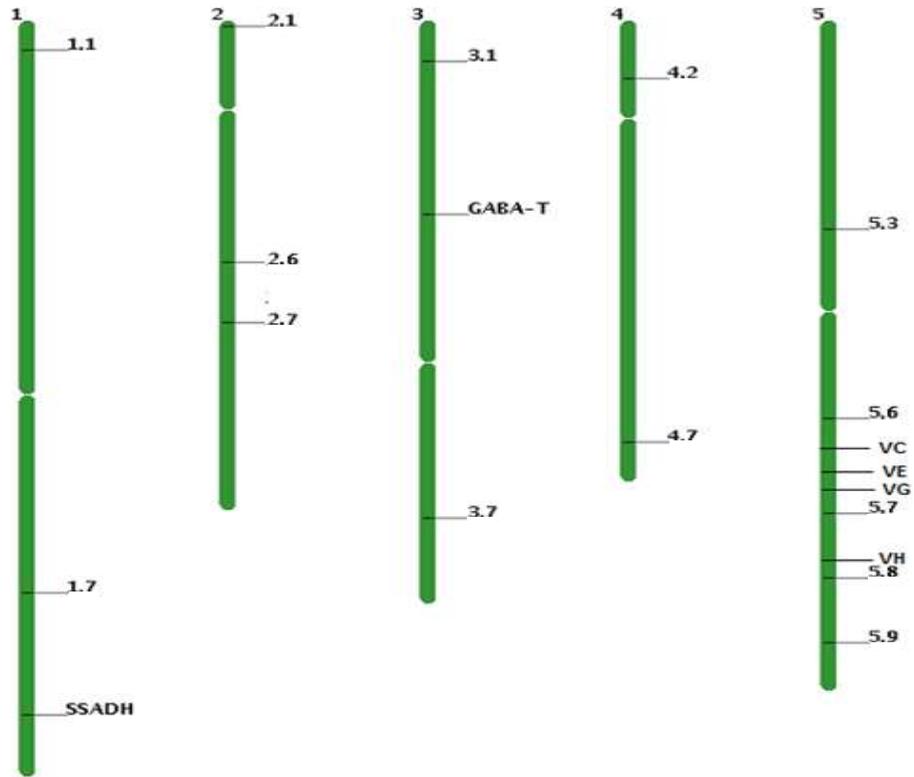


Figure S1. Schematic representation of the position of SCAR markers on Arabidopsis chromosomes.

Table S1. List of SCAR markers used in mapping of the EMS induced SNPs.

1.1-F	AACATCCAACGGTCTGAACC	Mapping
1.1-R	CGTGTCCGGTGAAAAAGAGT	Mapping
1.7-F	AAAACCCTGTTGTTTCTGAGC	Mapping
1.7-R	GGTTGAGACTGGTAACAAGG	Mapping
2.1-F	CCCATGCTTCCTCCTATTGC	Mapping
2.1-R	GGAGGCTCTTGAACCTCACAC	Mapping
2.6-F	TGTCACTGAAGAACCCTAGC	Mapping
2.6-R	GCAGCTTCGAGTGGATTCTA	Mapping
2.7-F	CTTCTTTCAAGGATCTCTTGC	Mapping
2.7-R	CCATGAATTCACCTCTCTATTC	Mapping
3.1-F	CACATTTTCAGTTATCTTAATGC	Mapping
3.1-R	GGAATGAGACATTGACTTC	Mapping
3.7-F	TGGGAACAAAGGTGTCATCC	Mapping
3.7-R	GCAAGTTAAAACCTGAAACTAAG	Mapping
4.2-F	GCTTTTAACCAGCTAACTTAGG	Mapping
4.2-R	GGTCTCTCACCTAAGGAGAT	Mapping
4.7-F	CCAATGATTGGTCACTACTGC	Mapping
4.7-R	GGTCATCAATTCATTTCTTAAGC	Mapping
5.3-F	CAGCTGCCTTCAAGTATTCC	Mapping
5.3-R	GCTGTGTTTTTGTAGAGAGCG	Mapping
5.6-F	CGTCAAAGACGACACATGG	Mapping
5.6-R	GCTCATGCTTCCTCCCATTG	Mapping
5.7-F	ATAAGATAGGTTTGGCAAATGG	Mapping
5.7-R	CCTACTATTCAAATTGTTTAAGAA	Mapping
5.8-F	CAAAAATTGATCGATCGATAGG	Mapping
5.8-R	CGTTATTGAGTCCGGTTGAG	Mapping
5.9-F	CTCTCGCTAACGCTCTTTGG	Mapping
5.9-R	GCACGAGTTAACGTTATTGAG	Mapping
VC-F	TCGGAAAAAGTATGTTGGGAGT	Mapping
VC-R	TGTTCAACAATAGCTGCCAAA	Mapping
VE-F	CCGATATAACTAAAGGTGCAGAGA	Mapping
VE-R	AAAATTCCTACCAAACGAAGCA	Mapping
VG-F	GATTTGCTCTCTGCCAAA	Mapping
VG-R	AAAAATGACGGGACGAAAGT	Mapping
VH-F	TCACCTTACTTAATTCAACTGCAA	Mapping
VH-R	CCAGATTCGATGTACTTCACTTTC	Mapping