Resistance determination of the ACCase-inhibiting herbicide of clodinafop propargyl in *Avena ludoviciana* (Durieu), and study of their interaction using molecular docking and simulation

Ali Akbarabadi1 · Ahmad Ismaili1 · Danial Kahrizi2 · Farhad Nazarian Firouzabadi1

Received: 26 July 2018 / Accepted: 10 November 2018 © Springer Nature B.V. 2018

**Abstract**

Structural mutations providing herbicide resistance may cause a modification of the three-dimensional structure of a protein which will lead to a decrease in the herbicide efficacy. Wild oat (*Avena ludoviciana* Durieu.) is an increasingly disruptive weed in areas of intensive cereal production, thus the aim of this research was to identify mutations conferring resistance to ACCase-inhibitor herbicides at greenhouse, laboratory and in silico scales. Among the selected biotypes, No. 3 in the position 1781 (Ile1781-Leu) and No. 14 in the position 2041 (Ile2041-Asn), showed resistance to ACCase-inhibitor. The above mutations were confirmed using the specific primers and PCR-based methods. Analysis of molecular docking indicated that residues of Trp1948 and Pro2001 are important in the binding site and showed remarkable variation in the mutation types. Using molecular dynamic simulation analysis, we demonstrated that mutation types changed the conformation of the enzyme. These changes resulted in compressed conformation in the active site, which limited the availability of binding herbicide-enzyme. In present, no crystallography molecular structure and modeling reported on the ACCase of plants and this study investigated interactions of clodinafop propargyl and ACCase CT domain in *A. ludoviciana* by modeling, docking and simulations for the first time. Totally, bioinformatics analysis as well as PCR-based method confirmed that herbicide resistance conferred by nucleotide mutations in the gene sequence.

**Keywords** Weed · Herbicide resistance · Point mutation · In silico · Binding energy · Modeling

**Introduction**

Wild oat is potentially harmful to crop yields due to staggered germination, production imitation, crop mimicry, a high degree of phenotypic diversity, high reproductive output, and seed persistence in the soil seed bank [1]. Until now, numerous attempts have been made to reduce weed biomass using herbicides and other approaches. However, populations of wild oat continue to persist, thus remaining the most abundant, competitive, and damaging weed in many areas [2]. There are currently about 494 herbicide-resistant weed biotypes worldwide, and this number continues to increase [3]. Aryloxyphenoxypropionate and cyclohexanedione herbicides inhibiting ACCase were both prevalent choices to manage the undesired traits of weeds, such as wild oat [4]. Frequent usage of herbicides like diclofop, fenoxaprop and clodinafop was also extremely harmful because of the high risk of herbicide resistance that is presently increasing rapidly all over the world [5].

ACCase is one of the essential regulatory enzymes of fatty acid biosynthesis, catalyzing the formation of malonyl-CoA from acetyl-CoA and bicarbonate in an ATP-dependent reaction producing the substrate for synthesis of fatty acids. Two isozymes of the ACC protein (ACC1 and ACC2) are available. Enzyme of ACC1, situated in the cytosol...
catalyzes the first step of fatty acid biosynthesis in lipogenic tissues. Enzyme of ACC2 that is bound to the mitochondrial membrane controls oxidation of fatty acid in oxidative tissue via allosteric modulation of carnitine palmitoyltransferase through production of malonyl-CoA [6, 7]. Isozymes of ACC contain two main domains, the carboxyltransferase (CT) domain and biotin carboxylase (BC) domain. Primary aims of inhibiting ACCase are focused on designing ligands that connect to the active site in the CT domain [8].

Two types of the mechanism are known in herbicide-resistance [5, 9]. The first group change the structure of the DNA sequence encoding the protein, i.e., structural mutations. The second group of mutations correlate with herbicide-resistance due to a variation in the expression of one or many genes in resistant plants compared with sensitive plants, i.e., regulatory mutations. Substitutions of Trp1999Cys, Trp2027Cys, Ile2041Asn, Asp2078Gly, Cys-2088Arg, and Gly2096Ser in the ACC1 gene were identified in Canadian populations of A. fatua [10], Powles and Yu [9], Zhang and Powles [11], Délye et al. [12] reported seven sites to endow ACCase-inhibitor resistance in various weed species among the thirteen conserved amino acid substitutions. These amino acid substitutions include Ile 1781-Leu or Val or Thr, Trp 1999-Cys or Leu or Ser, Trp 2027-Cys, Ile 2041-Asn or Val, Asp 2078-Gly, Cys 2088-Arg and Gly 2096-Ser or Ala. Substitutions of the amino acid at these seven positions showed various resistance patterns in the ACCase-inhibitors [9]. Délye et al. [13] using natural mutants of black-grass (Alopecurus myosuroides) by biological and molecular methods showed that residues Trp-2027, Asp-2078, and Gly-2096 induced insensitivity to ACCase-inhibitors. Also, they showed residues Trp-2027 and Asp-2078 are very likely to interact in the activity of CT domain.

The computational result structures of protein through modeling based on homology and threading, and by ab initio forecast, and docking of a protein structure with possible interacting partners are two relevant levels in computational proteomics. Molecular docking is an expression applied to computational projects, which assay to find the “best” matching between two molecules such as ligand and receptor. Molecular docking can determine two problems given the atomic coordinates of two molecules, and predict their “correct” bound association [14]. Molecular dynamics (MD) simulation is a simulation method, which allows the representation of interacting molecules of the atom in a definite time. The MD simulation technique is based on Newton’s law and classical mechanics. Lately, the advancement in unbiased structure prediction by MD simulation has been using brute force simulation methods, improved sampling algorithm according to metadynamics and other techniques [15–17]. Using molecular docking, Franca et al. [18] with the preferential orientation for the ACCase active site and diclofop and atrazine herbicides concluded that an inhibition coefficient of 0.168 mM for diclofop and 44.11 mM for atrazine was revealed. Also, result of this binding selectivity for the herbicide family of diclofop was confirmed by semi-empirical PM6 quantum chemical calculations, which show that ACCase interacts more strongly with the herbicide diclofop (with binding energies of −119.04) than with atrazine (with binding energies of +8.40 kcal mol⁻¹). Ramalho et al. [19] reported their results on molecular modeling by MD simulations and density functional theory (DFT) techniques. Afterwards, they discovered the interaction between glyphosate and its analogs with the wild type enzyme and Gly96Ala mutant EPSP synthase. They showed some important points in the design of new selective glyphosate derivatives. Zhang et al. [20] reported that construction of the inhibitor complexes of the CT domain exhibits a substantial conformational change in the active site of the enzyme that provides a highly conserved and very hydrophobic binding pocket that binds strongly into the dimer interface. Two residues are located on the binding site, which affect herbicide sensitivity; therefore, mutation in the mentioned residues disturbs the structure of the domain. Other residues in the binding site are conserved strongly in the CT domains. Délye et al. [13] via tridimensional (3D) modeling, reported that the side chains of the five residues are adjacent, and located at the surface of the inside of the cavity of the CT active site near the APPs binding site.

Herbicide resistance in the weed is a serious problem and there are a few in silico studies for the cause of weed resistance among populations at the molecular level. We asked the following questions about herbicide resistance in A. ludoviciana: (a) Can herbicide resistance in greenhouse be confirmed at molecular level by PCR-based technique? (b) Are mutations capable of changing active site of ACCase enzyme conformation? (C) Can mutations affect the binding of enzyme and herbicide?

Materials and methods

Greenhouse experiment

The wheat fields of Kermanshah province, west of Iran, were visited in 2015 to observe and collect samples of wild oat (A. ludoviciana) that survived the ACCase-inhibitor herbicide application. Sampled plants were dried and the seeds were harvested and bulked. Seventy populations with suspected resistance in A. ludoviciana were collected and each population was screened with the ACCase-inhibitor, clodinafop propargyl. In order to break seeds dormancy, seeds were incubated at 2–4 °C for 7 days, and then at 20 °C/16 °C (light/dark) for 4 days. The resulted seedlings were transplanted into pots and grown in a greenhouse with conditions...
at 25 °C/15 °C (day/night) with a 16-h photoperiod. The resistance level was determined through a greenhouse experiment. The clodinafop propargyl herbicide was used at 256 g a.i. ha⁻¹, which fully controlled the susceptible population. The experiment was conducted in a randomized complete block design with three replicates. Plant survival and herbicide symptoms were assessed 14 days after treatment. Plants were recorded as resistant if they displayed strong growth after herbicide treatment and as susceptible if they showed severe signs of leaf chlorosis, desiccation, retarded growth or plant death, as well as susceptible population [21]. The leaf tissues of resistant and susceptible (control) plants were harvested and stored in liquid nitrogen for a survey of molecular resistance.

**Molecular resistance**

Fourteen biotypes selected from the greenhouse experiment (13 resistant biotypes and one susceptible biotype as a control or wild type), were used to assay molecular resistance to ACCase-inhibitor herbicides. DNA was extracted as described by Doyle and Doyle [22] and kept at −20 °C prior to PCR analysis. All primers were originally designed based on the nucleotide sequence of CT domain of ACCase (ACC1) gene of *A. fatua* (Accession number KJ606970) (Table 1). In this research, the primers for a partial mRNA sequence of ACCase gene amplification by PCR were designed for six nucleotide point mutation probability position of 1781, 1999, 2027, 2041, 2078 and 2096 based on previous reports [13]. In addition, for confirmation of different point mutations in ACCase CT domain of *A. ludoviciana* (in wild and mutant biotypes), the resulted fragments from PCR amplification were purified from the agarose gel and sequenced. The nucleotide sequences were translated to amino acids and aligned with sequence of *A. fatua* CT domain.

**Modeling method**

The confirmed CT domain sequence of *A. ludoviciana* (that completely similar to partial amino acid sequence of the *A. fatua* ACCase CT domain protein with Accession Number A1B52367.1) was retrieved from the I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER) protein database, which has a sequence length of 412 amino acids. Presently, no high-resolution X-ray crystallographic structure for ACCase in plants is available; hence, 3D homology modeling may be an alternative to gain insights into potential substrate binding sites and the mechanisms of substrate interaction with the protein. Theoretical models of 3D structures of CT domain from susceptible and resistant biotypes designed using homology modeling and subsequent molecular dynamic simulation methods. The sequences of resistance and susceptible CT domain submitted to the I-TASSER server for comparative structural modeling. The corresponding sequences were aligned to identify the structural conserved region, and several suitable templates were selected. Table 2 shows the sequence identity between the selected templates. PROCHECK [23, 24] was used to check the stereochemical quality of these three homology models. All hydrogen atoms were generated to fill the unoccupied valence of heavy atoms at the neutral state in Insight II (2000). The output of server analyzed with a confidence score (C-score), the estimated template modeling score (TM-score) and RMSD (root mean square deviation) and the standard deviation of the estimates were used for confirming the 3D model.

### Table 1 Primers used to amplify the ACCase (ACC1) gene of *A. ludoviciana*

<table>
<thead>
<tr>
<th>PCR primer name</th>
<th>Sequence (5′–3′)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW1781</td>
<td>TGGACTAGGTGTGGAGAACA</td>
<td>303</td>
</tr>
<tr>
<td>FMC1781</td>
<td>GGACTAGGTGTGGAGAACCT</td>
<td></td>
</tr>
<tr>
<td>FMT1781</td>
<td>TGGACTAGGTGTGGAGAACC</td>
<td></td>
</tr>
<tr>
<td>RW1781</td>
<td>CTTCAAGGTCACTCTGGAGAC</td>
<td></td>
</tr>
<tr>
<td>FW1999</td>
<td>AGTTTTGTGGAGACATTTGAAG</td>
<td>200</td>
</tr>
<tr>
<td>RW1999</td>
<td>TTGGTACGTGAATCTGGAGAC</td>
<td></td>
</tr>
<tr>
<td>RM1999</td>
<td>CTGGTAGCTGAACTCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>FW2027</td>
<td>AGTTTTGTGGAGACATTTGAAG</td>
<td>282</td>
</tr>
<tr>
<td>RW2027</td>
<td>CCCACCAAGAAGCCTCTC</td>
<td></td>
</tr>
<tr>
<td>RM2027</td>
<td>CCCACCAAGAAGCCCTCTA</td>
<td></td>
</tr>
<tr>
<td>FW2041</td>
<td>CGATTGATCCAGCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>RW2041</td>
<td>CGATTGATCCAGCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>RM2041</td>
<td>CGATTGATCCAGCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>FW2078</td>
<td>AGTTTTGTGGAGACATTTGAAG</td>
<td>437</td>
</tr>
<tr>
<td>RW2078</td>
<td>CGATTGATCCAGCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>RM2078</td>
<td>CGATTGATCCAGCCTGAGAA</td>
<td></td>
</tr>
<tr>
<td>FW2096</td>
<td>AGTTTTGTGGAGACATTTGAAG</td>
<td>489</td>
</tr>
<tr>
<td>RW2096</td>
<td>TTGGACCTCGAGAACATTTG</td>
<td></td>
</tr>
<tr>
<td>RM2096</td>
<td>TTGGACCTCGAGAACATTTG</td>
<td></td>
</tr>
</tbody>
</table>

* F forward primer, R reverse primer, W wild type, M mutant

### Table 2 Results of ACCase CT domain enzyme modeling of *A. ludoviciana* with website online I-TASSER

<table>
<thead>
<tr>
<th>Model</th>
<th>C-score</th>
<th>RMSD (Å)</th>
<th>TM-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB model wild type</td>
<td>1.78</td>
<td>1.8 ± 2.3</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>PDB model Ile1781-Leu point mutant</td>
<td>1.79</td>
<td>1.8 ± 2.3</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>PDB model Ile2041-Asn point mutant</td>
<td>1.44</td>
<td>1.9 ± 2.6</td>
<td>0.96 ± 0.06</td>
</tr>
</tbody>
</table>

![Springer](https://link.springer.com)
**Molecular docking**

Specific site docking was carried out based on docking ligand into a protein target using AutoDock Vina (version 1.5.6) [25], with procedures documented in the previous studies [26]. The output structures thereafter were converted to PDBQT using MGL tools 1.5.6. The structures were also saved at their primary un-minimized energy states. All visualization of protein–ligand complexes were done using visual molecular dynamics (VMD) software [27]. The contents of 3D macromolecular structure were calculated by PDBsum to generate (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) web-based server [28] and C-Finder (http://bioinf.modares.ac.ir/software/cfinder/) server. Energy minimization calculations carried out by GROMACS macromolecular simulation software.

**Molecular dynamics simulation**

The MD simulations utilized in this study to determine the most appropriate conformations of the ACCase in an aqueous state of the enzyme are investigated. The molecular simulation was carried out using the GROMACS package (version 5.0.1) [29]. The GROMACS is one of the world’s fastest software packages for MD simulations. Crystallographic water molecules were maintained and the two systems (enzyme and enzyme–herbicide complex) were solvated in a bath of SPC (Statistical Process Control) water molecules [30]. To maintain the systems at a reference temperature of 300 K, the modified Berendsen thermostat was employed by a coupling time of 0.1 ps. The pressure was maintained by coupling to a reference pressure of 1 bar [30]. Molecular dynamics simulations of bulk water were used at a constant time of 0.1 ps. For water simulations, the value of the isothermal compressibility of water was set to $4.5 \times 10^5 \text{ bar}^{-1}$. All the bond lengths (even heavy atom-H bonds) were constrained by the LINCS (Linear Constraint Solver) algorithm [31]. The electrostatic interactions were evaluated using the Particle Mesh Ewald method [32]. Analyses of the trajectories were generated from the molecular dynamics simulations performed by GROMACS (version 5.0.1) programs. Molecular graphics were made with the VMD program [27].

**Results**

**Field sample analysis**

The survival capacity and susceptibility of biotypes were evaluated in the greenhouse under the above-mentioned condition. The greenhouse study confirmed resistance to ACCase inhibiting herbicide in 18.5% of biotypes (13 biotypes among 70 suspect biotypes).

**Allele-specific PCR amplification**

PCR was performed using wild type forward primer (FW1781) and wild type reverse primer (RW1781) (Table 1) for 14 biotypes. The results indicated that the lack of bands for biotype No. 3 (Fig. 1) contained a single nucleotide mutation. According to the analysis, other biotypes did not show this point mutation. Thus, for denotation mutation nucleotide A to T/C, PCR based on forward primer mutant T (FMT1781) with wild type reverse primer (RW1781) (lane 1, 2 and 3 in Fig. 2) as well as forward primer mutant C (FMC1781) with wild type reverse primer (RW1781) (lane 4, 5 and 6 in Fig. 2) in three repeats was conducted. Fragment was favorable according to mutant primer T (FMT1781) with a 303 bp length observed, which conferred mutation nucleotide A to T (ATA to TTA) in the first codon position. In addition, the resulted fragments of PCR amplification from wild and mutant biotypes were sequenced and the obtained sequences confirmed the results of PCR.

Results of PCR using wild type forward primer (FW2041) and wild type reverse primer (RW2041) for 14 biotypes showed the lack of band for Biotype No. 14 (Fig. 3). For denotation mutation in the position 2041 for Biotype No. 14, PCR based on wild type forward primer (FW2041) and mutation reverse primer (RM2041) was

![Fig. 1](image-url)"
conducted in four repeats (Fig. 4). The result indicated that mutation by substitution in the second codon position (ATT to AAT) led to conversion of Ile2041 to Asn in the ACCase gene of *A. ludoviciana*, which probably confers resistance to the clodinafop propargyl herbicide. The resulted fragments of PCR amplification from wild biotype and mutant biotypes were purified from agarose gel and were sequenced. The obtained sequences confirmed the results of PCR.

Results of allele-specific PCR amplification using other primers (wild type forward primer and wild type reverse primer) (Table 1) showed amplification of specific target bands for all 14 biotypes (data not shown). Thses results indicated that there were no mutations in biotypes for these five point mutations.

**Analysis of modeling and molecular docking**

The results of modeling showed that modeling is well presented based on factors of the C-score, TM-score, RMSD and B-factor (Table 2). The C-score is the confidence score of the prediction. C-score ranges [−5 to 2], where a higher score indicates a more reliable prediction and demonstrates a strong correlation with the real quality of the final models. The RMSD was used as a quantitative measure of similarity between two or more protein structures [33]. All factors showed the accuracy of the modeling structure for the ACCase enzyme in *A. ludoviciana*; therefore, 3D structure enzyme can be examined to assess docking.

The ligand tested in multiple conformations including wild type and 2 point mutation (Ile1781-Leu and Ile2041-Asn). In the wild type, positions of Thr1861, Asn1862, Gly1947, Trp1948, Ala1949, Lys1950, Gln1976, Leu1977, Val1978, Arg1994 and Asp2002 were involved.
in the binding with herbicide (Fig. 5a). The mutation type changed the conformation of the ACCase enzyme and created binding positions on the surface of the CT domain. These positions in the Ile1781-Leu point mutation include Asn1862, Trp1948, Ala1949, Gln1976, Val1978, Val1992, Arg1994, Pro2001, Asp2002, Thr2005, Glu2039, Gln2043 and Ala2044 (Fig. 5b), and in the Ile2041-Asn point mutation include Ser1877, Leu1880, Arg1881, Ser1884, Tyr1885, Thr1897, Ser1899, Leu1900, Asp1937, Gln1938, Asp1939, Ser1940 and Lys1958 (Fig. 5c).

Molecular docking was carried out to survey the binding mode of ACCase enzyme with herbicide in the wild and mutant types (Tables 3, 4, 5). In the wild type, the oxygen atom of Asp2002 with the N1 position of 24 showed hydrogen and ionic interactions (electrostatic) with binding energy −9.4 and −3.2 kcal/mol, respectively (Table 3). These residues are important for the interaction and binding of clodinafop propargyl herbicide on the ACCase enzyme, while in the Ile1781-Leu point mutation, Trp1948 and Pro2001 positions (Table 4) and in the Ile2041-Asn point mutation, Asp1939 position (Table 5) showed ligand-receptor interaction.

Molecular docking was carried out to survey the binding mode of ACCase enzyme with herbicide in the wild and mutant types (Tables 3, 4, 5). In the wild type, the oxygen atom of Asp2002 with the N1 position of 24 showed hydrogen and ionic interactions (electrostatic) with binding energy −9.4 and −3.2 kcal/mol, respectively (Table 3). In the Ile1781-Leu point mutation, the oxygen atom of Pro2001...
with the N1 position of 24 demonstrated H-donor interaction with binding energy $-1.7$ kcal/mol, and the N atom of Trp1948 with 6-ring ligand indicated H-acceptor interaction with binding energy $-0.8$ kcal/mol (Table 4). In the Ile2041-Asn point mutation, the oxygen atom of Asp1939 with the N9 position of 22 showed H-donor interaction with binding energy $-1.6$ kcal/mol (Table 5).

**Analysis of simulation**

The RMSD of the wild and mutant types of ACCase enzyme after a mass-weighted superposition on the starting structure was calculated (Fig. 6). The RMSD becomes stable at about 24 ns for wild and mutation types. Simulation was equilibrated and its fluctuations towards the end of the simulation were around some thermal average structure. The RMSD relative to the starting structures of both sets of independent simulations stabilizes quickly after the release of the constraints on the heavy atoms [34]. The root mean square fluctuation (RMSF) is useful for characterizing local changes along the enzyme. According to the RMSF of three enzymes, mutation type enzymes were more compact than the wild type enzyme (Fig. 7). This means that the less molecular surface of the mutation type enzyme was exposed to the herbicide. According to the results of these analyses, it shows that the enzyme has been stable and hence, further analysis can be made on the simulation pathways.

The simulation results showed that the binding energy of the wild type is more stable than the mutant types (Table 6), which is consistent with the experimental results. In addition, with the help of this analysis, we can estimate the contribution and characteristics of each of the interactions. Interaction energy in the wild type is $-56.7$ kcal/mol, while in the Ile1781-Leu and Ile2041-Asn point mutant are $-21.11$ and $-24.78$ kcal/mol, respectively (Table 6). The electrostatic energy and van der Waals energy in the wild type were stable, whereas in the other two mutants were more unstable.

Amount of energy released in the wild type is more than that of the mutation types. In the wild type relative to mutation types, stronger connection was created between the ACCase enzyme and herbicide, which led to the more stable interaction. Therefore, this strong binding between herbicide and ACCase enzyme changed the enzyme activity and caused plant death.

**Discussion**

The greenhouse study confirmed resistance to ACCase inhibiting herbicide in 18.5% of biotypes. Beckie et al. [35] demonstrate that ACCase-inhibitor resistance to wild oat evolves much more slowly in perennial versus annual cropping systems. Cruz-Hipolito et al. [36] reported resistance to the three-herbicide families in the biotypes of wild oat (Avena fatua) from Chile based on dose–response and ACCase enzyme activity.

We have demonstrated nucleotide substitution Ile1781-Leu and Ile 2041-Asn to ACCase-inhibiting herbicide for biotypes No. 3 and 14, respectively. Substitution of Ile1781-Leu was identified in weeds of L. rigidum [37] and S. uiridis [38], while the other possible leucine ACCase alleles were found in A. myosuroides [39]. Resistance based on target site typically was created through a single amino acid change.

![Fig. 6](image)

**Fig. 6** Time evolution of the root mean square deviation (RMSD) of molecular dynamic structure with respect to the corresponding minimized structures wild type of acetyl coenzyme A carboxylase (ACCase) enzyme and mutant models during 30 ns molecular dynamic simulation time. Ile1781-Leu point mutation: substitution isoleucine 1781 with leucine; Ile2041-Asn point mutation: substitution isoleucine 2041 with asparagine.
in the CT domain (Ile1781-Leu and Ile2041-Asn), which influences the productive binding of ACCase-inhibitors. Mechanism of resistance in another biotype that didn’t show point mutation by the PCR-based method may be due to non-target-site resistance. In these biotypes, expression of herbicide metabolizing enzyme(s) or transporter proteins will lead to an increase in herbicide degradation or compartmentation away from its site of action [40]. In addition, resistance in the other biotypes may be caused by mutations which has occurred in another location of the coding gene of ACCase enzyme.

De’lye et al. [41] reported that ACCase CT domain was highly conserved both between and within grass species. Although Linda et al. [42] and Zhang et al. [20] reported X-ray crystallographic structure for yeast ACCase, the similarity of amino acid sequence of yeast and oat plant is low (about 52%). In present, no crystallography molecular structure and modeling have been performed on the ACCase of plants. Hence, 3D homology modeling may be an alternative to gain insights into potential substrate binding sites and the mechanisms of substrate interaction with the protein. The details of the interaction between ligand-receptor were investigated via computational methods such as molecular docking and MD simulation. For this purpose, we have first generated an ACCase 3D model by online I-TASSER tool. The output of the I-TASSER server for ACCase enzyme modeling was confirmed with C-score, TM-score, RMSD and the standard deviation of the estimates. The desired orientation of the ACCase-inhibitor with the active site of the ACCase enzyme of *A. ludoviciana* was determined by molecular docking calculations, which displayed an inhibition for clodinafop propargyl herbicide. The charged residue (Ile1781-Leu and Ile2041-Asn) is commonly found in the active sites of carbohydrate-binding proteins of ACCase [10, 43, 44]. There are significant conformational changes in the protein conformers due to the mutations.

For the “binding energy/binding affinity”, the more negative energy is better to connect ligand-receptor. In the wild type, the binding energy of Asp2002 is −9.4 kcal/mol, and is more negative, showing that binding affinity is adaptable. Since the binding energy was more negative, the binding of the receptor-ligand was carried out with a minimum amount of energy. Thus, amino acid Asp2002 plays a

![Graph showing RMSF difference between wild type and mutant models of acetyl coenzyme A carboxylase (ACCase) enzyme](image-url)

**Table 6** The energy result of interaction of clodinafop propargyl herbicide and ACCase enzyme after simulation. vDW: van der Waals energy

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Interaction energy (kcal/mol)</th>
<th>Electrostatic energy (kcal/mol)</th>
<th>vDW energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>− 56.7</td>
<td>− 38.23</td>
<td>− 24.53</td>
</tr>
<tr>
<td>Ile1781-Leu point mutant</td>
<td>− 21.11</td>
<td>− 13.62</td>
<td>− 3.45</td>
</tr>
<tr>
<td>Ile2041-Asn point mutant</td>
<td>− 24.78</td>
<td>− 17.67</td>
<td>− 8.88</td>
</tr>
</tbody>
</table>

1781 with leucine; Ile2041-Asn point mutation: substitution isoleucine 2041 with asparagine
very important role in the interaction of herbicides with the ACCase enzyme. Conformational changes in the structure of this enzyme imply that interaction forces between herbicide and mutated enzyme can be weakened in reactive site, which led to poor force propagation and incorrect diagnosis. The results of the present research showed that hydrogen bond between receptor-ligand in the wild type plays an important role in the binding of herbicide to the enzyme.

The structural fluctuations and the differences between ACCase enzyme in the wild-type and mutant may be dependent on the two important factors: (1) electrostatic interaction between charged amino acids such as glutamic acid, aspartic acid, arginine and lysine, (2) increasing mobility of protein due to hydration of the polar amino acids [18]. A latter approach is a powerful tool for examining the macromolecule solvation because it can provide a description at the atomic level and at the appropriate timescale. Based on simulation analysis, the best energy conformation was evaluated for the binding free energy. Calculated energies of simulation and derived docking receptor-ligand for the desired complexes are in accordance with Table 6. This result showed that binding herbicide-enzyme in the wild type became stable more than the mutant types.

Binding energy in the positions Trp1948 and Pro2001 in Ile1781-Leu point mutation and in the position Asp1939 in Ile2041-Asn point mutation were very poor. Therefore, the herbicide should spend more energy to connect to the enzyme. Our results showed that the mutations of Ile1781 and Ile2041 lead to sites with different shapes, surfaces and properties, which might make ACCase-inhibitors resistance. Choe et al. [45] indicated that single point mutants Pro197-Thr, Pro197-Ala or Pro197-Ser showed significant conformational changes in the pyruvate-binding pocket of acetohydroxy acid synthase (AHAS).

Conclusion

Mechanism of target site resistance is known as the predominant mechanism of resistance to ACCase-inhibiting (clodinafop propargyl) herbicides in A. ludoviciana biotypes [46]. In current research, the PCR-based method confirmed Ile1781-Leu and Ile2041-Asn substitution to ACCase enzyme resistant of clodinafop propargyl in A. ludoviciana.

There are few reports on modeling, docking and simulation about interaction of herbicides and their substrates in plants. ACCase-inhibiting herbicides are one of the most important herbicides families that their mechanisms of action are different from other herbicides families. Although previous studies reported X-ray crystallographic structure and modeling in yeast ACCase, there was no report on modeling, docking and simulation of ACCase in plants. Our study investigated interactions of clodinafop propargyl and ACCase CT domain in A. ludoviciana by modeling, docking and simulations for the first time. The results of molecular docking and MD simulation analysis, as well as PCR-based method showed that herbicide resistance conferred by nucleotide mutations in the gene sequence. The mutations: Ile1781-Leu and Ile2041-Asn cause significant structural changes in the shape, surface and property of domain located in the N-terminal of ACCase enzyme. Mutation of Ile1781 and Ile2041 gives rise to broad cross-tolerance among ACCase-inhibitors and ACCase enzyme. The biochemical activity of ACCase enzyme is strongly associated with Asp2002 and Trp1948 both of which play an important role in the binding of herbicide to the enzyme. It is recommended that future researchers should consider that the above-mentioned residues may be regarded as important amino acids to study herbicide resistance or non-resistance to ACCase inhibitor.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References


