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# Diagnosis of mutations in the ALS and ACCase genes in Loluim weed

**By**

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## Summary

During the winter agricultural season 2021-2022, a field experiment was conducted in one of the fields located in the Al-Hindiyah District of the Holy Karbala Governorate. The purpose of the experiment was to study potential mutations that could make plants resistant to the action of certain herbicides belonging to the genus Lolium. The experiment was designed according to the randomized complete block design (R.C.B.D) with split plots arrangement and three replications, as the main plots included four different types of herbicides, namely Chevalier, Pallas, Tobik, and Axial, along with a comparison treatment (spraying with water only), while the sub plts included planting Lolium bush seeds. The holy province of Karbala, the province of Babylon, and the province of Wasit are the three provinces that make up this grouping in the Iraqi country of Iraq. The results revealed mutations in the ALS gene in the mRNA sequences of plants from Karbala, Wasit, and Babylon that are resistant to the pesticides Chevalier and Pallas, by substituting nitrogenous bases with other bases in codons 195 and 200 of AGC and AGT, which encode for the amino acid Ser to ACC, and GGT, which encodes for Thr and Gly. Iraqi weed mutations matched global mutations. Two missense mutations were found in codons 175 and 266 of the succession of Wasit governorate plants, by substituting codons GTC and CGC, which encode for Val and Arg, by codons GGC and TGC, which encode for Gly and Cys. The results of this gene in the weed (Lolium temulentum) were identical to the weed Lolium rigidum, except for one silent mutation at codon number 150 GCT that encodes for the amino acid Ala. This codon was changed to GCC in the weed of Wasit province. The results revealed a number of mutations resistant to the action of the two pesticides, Tobic and Axial, in the ACCase gene in the mRNA sequences of the plants of the Holy Karbala, Wasit and Babel governorates. These mutations are similar to the mutation in the resistant plants recorded globally, by substitution of nitrogenous bases with others in codons 25, 29, 33, 167, and 209 from CGA, GAA, AAT, TTG, and ACA, These mutations in the Iraqi weed coincided with mutations recorded globally, and the results showed that there is a missense mutation in codon 161 of the sequence of Wasit and Karbala pesticide-resistant plants, by substituting two nitrogenous bases from AAG, which encodes Lys, to AGA, which encodes Arg. In Karbala and Wasit, pesticide-resistant plants had two frame shift mutations.

**Keywords:** Herbicides, Loluim weed , ALS, ACCase, mutations.

# Introduction

Due to the vital role it plays in ensuring that people all over the world have access to sufficient food, the wheat crop (Triticum aestivum L.) currently holds the number one production spot in the world. Although Iraq was one of the first regions in the world to see the rise of wheat, the country's overall wheat productivity is still quite low [ 1 ].The weed is considered the most important vital factor affecting agricultural production because it is responsible for losses ranging from 15–65% depending on the density and type of weed as well as the period that it is present. One of the most important reasons for the low productivity of this crop is the weed, as it is considered the most important vital factor affecting agricultural production [ 2 ]. As a result, those engaged in agriculture and researchers sought to combat it in a variety of ways, including the employment of chemical treatments due to the convenience of their application and the speed of their effect [ 3 ].The weed known as Lolium is regarded as a noxious pest on a global scale and is responsible for major losses in grain productivity. There have been 17 species described, and these species are able to interbreed with one another, which can result in individuals who are either homozygous or heterozygous, both of which are able to carry multiple resistance alleles [ 4 ].Herbicide resistance is defined by the American Weed Science Society (WSSA) as the hereditary ability of particular biotypes within a species to survive and reproduce at a given dose of herbicides with which the original species was killed. This ability is passed down from generation to generation. Herbicide-resistant weeds are often the result of a single mutation occurring inside a gene. This mutation causes a structural change in the active sites of the enzyme to which the herbicide binds, rendering the herbicide less effective against the weed. This alteration lessens or eliminates the binding that occurs between the herbicide and the enzyme that is the target, or it does so through mechanisms that reduce the amount of active herbicides that reach the target site. These mechanisms include improved metabolism, a decreased transport rate of herbicides, and isolation of herbicides from the enzyme that is the target [ 5 ] .It was explained by [ 6 ] .that the Lolium weed is difficult to control chemically because of its ability to develop different resistance mechanisms to herbicides very quickly. In addition to this, the repetition of planting in the same crop causes natural selection of weeds that are resistant to herbicides, which in turn causes changes in the types of weeds that are found in the region as well as the reproduction of plants that are less sensitive to herbicides. The trait of resistance is one that has developed over the course of evolutionary history. Herbicides such as ALS inhibitors and ACCase inhibitors have the ability to select resistant biotypes within one to five election cycles, which renders the continued use of herbicides worthless and leads to a rise in resistance [ 7 ] .Target site resistance refers to a mutation that occurs in genes that encode target enzymes and can cause weeds to be resistant to herbicides such as ALS and ACCase-inhibiting herbicides (TSR). In most cases, resistance to particular herbicide compounds is conferred on the weed L. rigidum by mutations at specific locations in the ALS or ACCase genes L. rigidum [ 8 ]. The most prevalent mutations associated with weed resistance to herbicides containing sulfonylureas are those that include a change in the amino acid that is located at position Pro197 and Trp574 in the ALS enzyme [ 9 ]. In the ACCase enzyme, amino acid substitutions at positions Ile1781, Trp2027, Ile2041, and Asp-2078 have been found to frequently confer resistance to aryloxyphenoxypropionate (APP) and cyclohexanedione (CHD) cyclohexanedione [ 10 ,11 ]. This resistance allows the organism to avoid the effects of the herbicides.

In plant species and microorganisms, ALS is the primary enzyme responsible for the production of the branched-chain amino acids leucine, valine, and isoleucine [ 12 ] .Over-reliance on ALS-inhibiting herbicides in crop systems has led to the development of ALS-inhibitor resistance in more than 159 noxious weeds worldwide [ 13, 14 ]. Since the early

1880s, ALS-inhibiting herbicides have been widely used for weed management in agricultural fields throughout the world [ 15 ] .

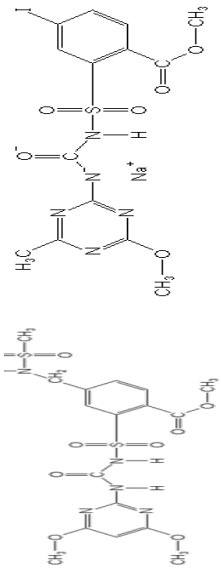
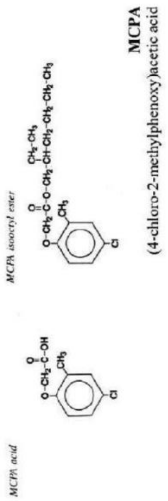
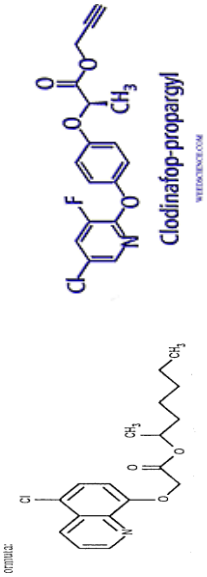
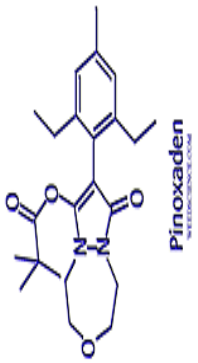
Since their introduction in the middle of the 1970s, ACCase inhibitors have been the compounds of choice for weed management. They are the most cost-effective and efficient means for controlling weeds in wheat, and they have been in use ever since [ 16 ] .ACCase is the first enzyme in the route that leads to the production of fatty acids (valine, leucine, and isoleucine), and it acts as an inhibitor. Helical hexanes (DIMs), aryloxyphenoxypropionate (FOPs), and (DEN) phenylpyrazolin are the three distinct chemical classes that ACCase belongs to[ 17 ].

## Materials and Methods

In the duration of the winter agricultural season 2021-2022, in one of the fields located within the Al-Hindiya District of the Holy Karbala Governorate, an experiment was conducted in order to investigate the possibility of mutations within certain weeds belonging to the genus Lolium that would make them resistant to herbicides. During the course of the season, seeds of various weeds belonging to the genus Lolium were gathered from a variety of areas across Iraq, specifically in the governorates of Karbala, Babel, and Wasit. Agricultural 2020-2021, and it will be stored until the time that the experiment is actually carried out. In the weed laboratory at the College of Agriculture, Karbala University, samples collected from three different governorates in Iraq were analyzed for the presence of various weeds. The experiment was conducted using a split plot design with three replications, and the main plots contained four different kinds of herbicides: Chevalier, Pallas, Tobik, and Axial. The design of the experiment was based on a split plot. Herbicides were sprayed on the weed in accordance with the recommendations provided by the manufacturer (Table1). Regarding the secondary plates, they contained three different groups of weed seeds belonging to the genus Lolium. These seeds were gathered from three different governorates in Iraq: Holy Karbala Governorate, Babel Governorate, and Wasit Governorate.

**Table 2:** *Study herbicides and spraying times.*

| The name of the herbicides | Chevalier | Pallas | Tobik | Axial |
|---|---|---|---|---|
| group | Sulfonylurea | Triazolopyramidine Sulfonamide | Aryloxyphenoxypropionate (s (FOPs | Phenylpyrazoline |
| concentration | $H^3$ 300gm | 500-450 gm $H^3$ | gm $H^3$ 240-200 | gm $H^3$800-600 |
| Effective Material | Iodosulfuron - methyl + Mesosulfuron – methyl | Pyroxsulam | CLODINAFOP-PROPARGYI | DICLOFOP METHYI |

| | | | | |
|---|---|---|---|---|
| **Synthetic summer** |  |   |   |   |
| **spraying stage** | When the bush is in the stage of (3-6) leaves | When the bush is in the stage of (3-6) leaves | It is used when the bush reaches the stage (3_5) leaves | It is used when the bush reaches the stage (3_5) leaves |
| **The type of pesticide** | My device is optional and is mildly toxic | My device is optional and is mildly toxic | My device is optional and is mildly toxic | My device is optional and is mildly toxic |
| **target bushes** | Thin bush and broad leaves | Thin bush and broad leaves | Thin-leaved bush | Thin-leaved bush |

## Diagnosis of ALS and ACCase genes

***RNA extraction***

The equipment prepared by the American company Bioneer, whose components are attached in Table2, was used for the purpose of extracting RNA from weed Lolium leaves for three Iraqi governorates, Babel, Karbala, and Wasit.

**Table2:** *Components of the RNA extraction kit provided by Bioneer.*

| volume (µl) | the ingredients | S |
|---|---|---|
| 30 | RB Buffer (RNA) | 1 |
| 40 | Washing) RWA1 Buffer (1st | 2 |
| 70 | RWA2 Buffer (2nd Washing) | 3 |
| 10 | ER Buffer (Elution) | 4 |
| 50 | Binding Column- III AccuPrep. | 5 |
| 50 | Collection for 1.5ml tupe | 6 |

***Reverse transcription RT PCR method***

RNA samples were extracted from the leaves of three Iraqi provinces: Karbala, Babel, and Wasit. Reverse transcription polymerase chain reaction (RT PCR) was carried out on these samples using the 2 X AddScript RT-PCR SYBR Master kit (Table3), and in the presence of

specialized primers for the diagnosis of ALS and ACCase genes (Table4). The final volume was supplemented with water (Nuclease-free water) to 25 microliter.

**Table3:** *Components of the RT-PCR mixture and their respective*

| (volume (µl | the ingredients | S |
|---|---|---|
| 10µl | 2 X AddScript RT-PCR SYBR Master | 1 |
| 2µl | Forward primer | 2 |
| 2µl | Reverse primer | 3 |
| 4 µl | RNA | 4 |
| 2µl | Distill water | 5 |
| 20µl | final size | |

***Concentrations***

**Table4:** *Primers specific for diagnosing ALS and ACCase genes used in RT PCR*

| initiator symbol | initiator symbol | sequencing | Tm (°C) | GC (%) | output volume bp |
|---|---|---|---|---|---|
| ALS | ALS1 | F: 3' CAACTGCCACTTCGACAGC '5<br>R: 3' CGAGGACGAGGTAGTTGTGC '5 | 60.6<br>60.9 | 57.9<br>60 | 585 |
| | ALS 2 | F; 3' CTCCATCACCAAGCACAACT 5'<br>R; 3' GCTGCTTGTTCTTGCCAATC 5' | 58.7<br>60.9 | 50<br>50 | 572 |
| | ALS3 | F; 3' AAGCAGGTCCAAGATTGTGC 5'<br>R: 3' TCCAAGATGCTGGTTGTTCA 5' | 60.3<br>60.2 | 50<br>45 | 550 |
| | ALS4 | F: 3' GCATTGAGAACCTCCCAGTT 5'<br>R: 3' ATACGCAATCCTGCCATCAC5' | 59.1<br>60.9 | 50<br>50 | 356 |
| ACCase | ACCase1 | F;3'AACTGGGTATTCTGCCCTGA 5'<br>R;3'CTGCGCTGTCTTGGTAGCAG 5' | 60.1<br>59.9 | 50<br>50 | 565 |
| | ACCase2 | F; 3'AACTGGGTATTCTGCCCTGA 5'<br>R; 3'CTGCGCTGTCTTGGTAGCAG 5' | 59.5<br>62.2 | 50<br>60 | 532 |
| | ACCase3 | F; 3' CACAGACCATGATGCAGCTC 5'<br>R; 3' GGCAGCAACTGTTTCTTTCG 5' | 60.4<br>60.9 | 55<br>50 | 571 |

The reaction mixture was prepared in a sterile tube (a tube for each gene with a negative control), and its components were mixed using a fine pipette, then placed in a centrifuge to maintain the final volume of the reaction mixture, then it was placed in the instantaneous thermopolymerization device, and the programs shown in Tables 4 were implemented. and 5 for the purpose of amplifying the ACCase and ALS genes, respectively

**Table5:** *Program RT PCR conditions for ACCase1 gene amplification.*

| stage | temperature (°C) | the time | The number of courses |
|---|---|---|---|
| cDNA synthesis | 50 | 20 min. | Hold |
| Initial Denaturation | 95 | 10 Min | Hold |
| Denaturation | 95 | 30 sec | |
| Annealing | 58 | 30 sec | |
| Extension | 72 | 1min. | 35 |
| Extension | 72 | 5min. | Hold |

**Table 6:** *Program RT PCR conditions for ACCase2, ACCase3, ALS1, ALS2, ALS3, and ALS4 gene amplification.*

| stage | temperature (°C) | the time | The number of courses |
|---|---|---|---|
| cDNA synthesis | 50 | 20 min. | Hold |
| Denaturation Initial | 95 | Min10 | Hold |
| Denaturation | 95 | 30 sec | 35 |
| Annealing | 60 | 30 sec | |
| Extension | 72 | 1min. | |
| Extension | 72 | 5min. | Hold |

After the RT-PCR process was finished, the samples were put through electrophoresis. Following that, the agarose gel layer that contained the double-stranded nucleic acid products was analyzed using ultraviolet radiation, and images were made of it.

***Sequence analysis of cDNA.***

In order to determine the sequence of the nitrogenous bases of the complementary nucleic acid products duplicated by reverse transcription polymerase chain reaction (RT PCR) for weed samples and for the identified genes, the complementary nucleic acid products were sent to the Korean company Macrogen along with the forward primer that was used in the process of DNA replication. This was done so that the sequence of the nitrogenous bases of the complementary nucleic acid products could be determined (Table3) After that, the sequence of the nitrogenous bases of the double-stranded DNA product was entered into the database that is accessible at the National Center for Biotechnology Information (NCBI), and the Bio Edit program was used to match the sequences of the nitrogenous bases with each other. NCBI PLAST was utilized in order to sketch the genetic tree.

# Results

***Diagnosis of the ALS gene***

Following the step of establishing the optimal conditions for the RT-PCR reaction, the products of the reaction were then transferred to an agarose gel. The findings presented in Figures 1, 2, 3, and 4 demonstrated the existence of bands with sizes of 585, 572, 550, and 356 base pairs in the weed samples that are both sensitive and resistant to the effects of herbicides, as well as in the samples from the three governorates (Babylon, Karbala, and Wasit), which together represent the spectrum of herbicide sensitivity. An enzyme that catalyzes the first reaction in the biosynthesis pathway of the branched chain amino acids valine, leucine, and isoleucine is encoded by the ALS gene, which is responsible for herbicide resistance. This process is necessary for the production of the branched chain amino acids.

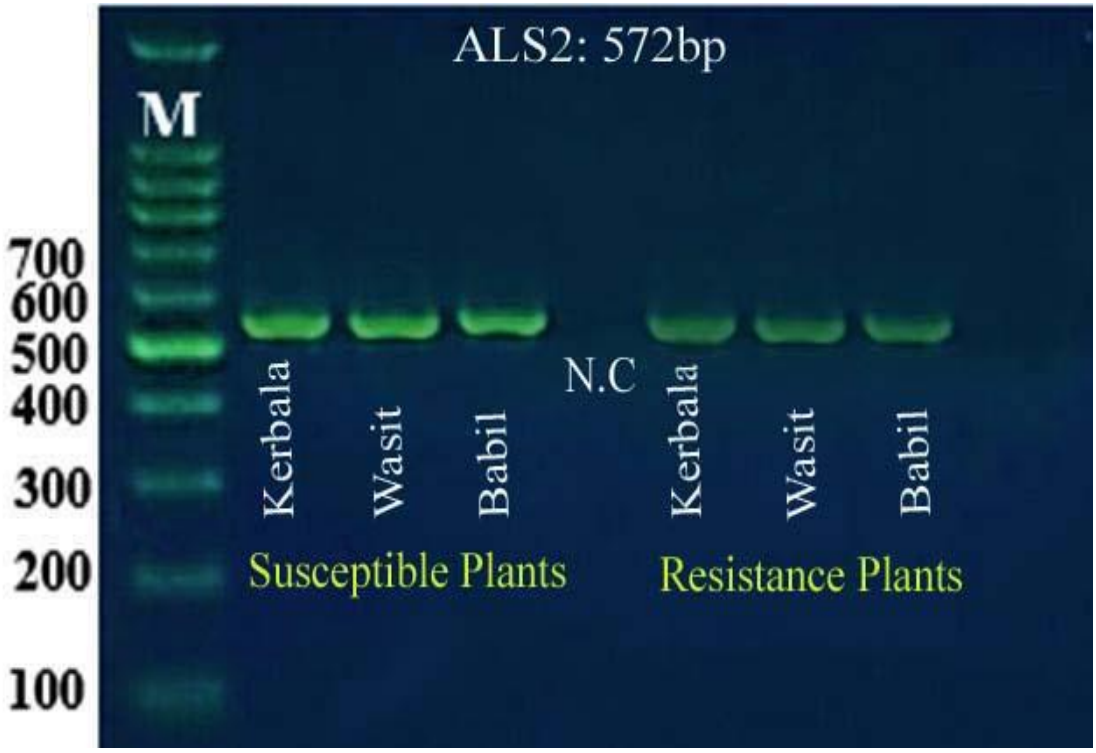



**Figure1:** *Electrophoresis of ALS1 gene amplification data for sensitive and resistant plants to Chevalier and Pallas pesticides in three Iraqi provinces with a negative sample (N.C).*

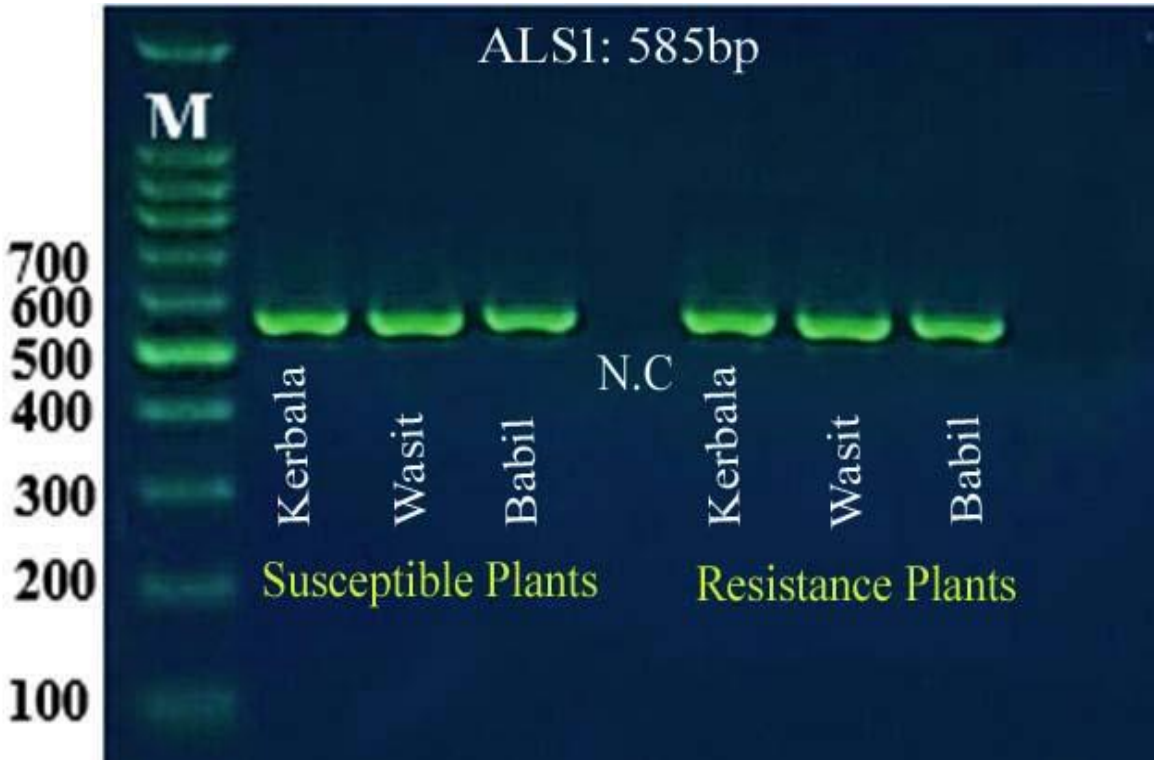



**Figure 2** *Electrophoresis of 2ALS gene amplification of plants sensitive and resistant to Chevalier and Pallas in three Iraqi provinces after 45 minutes on 1% agarose gel at 95 volts.*


**Figure 3** *Electrophoresis of 3ALS gene amplification of sensitive and resistant plants to Chevalier and Pallas pesticides in three Iraqi governorates after 45 minutes on 1% agarose gel at 95 volts.*

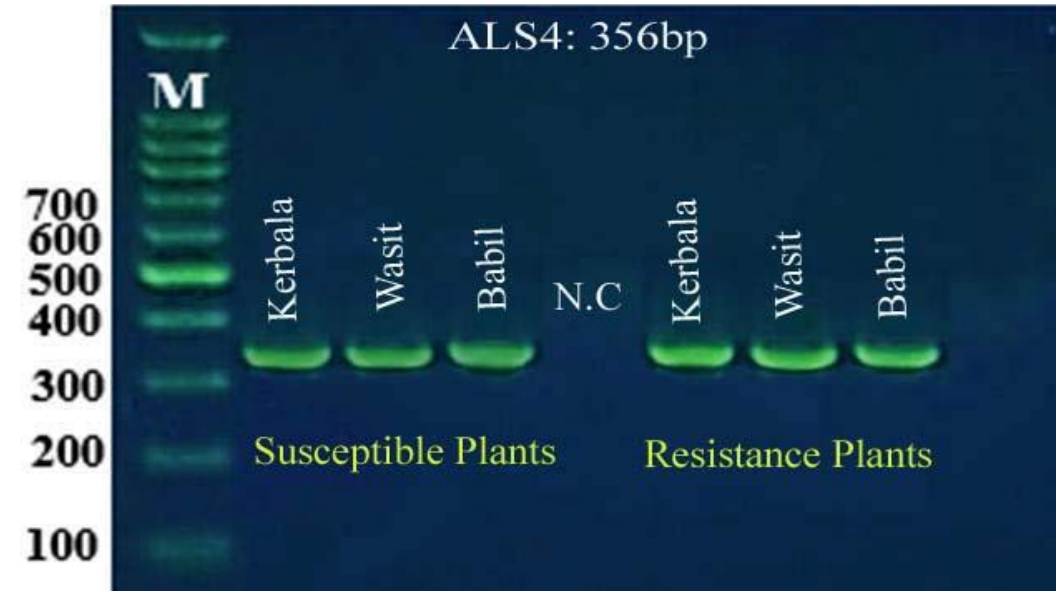



**Figure 4:** *Electrophoresis of sensitive and resistant plants to Chevalier and Pallas pesticides in three Iraqi provinces, after electrophoresis on 1% agarose gel at 95 volts for 45 minutes.*

## Diagnosis of the ACCase gene

Following the step of establishing the optimal conditions for the RT-PCR reaction, the products of the reaction were then transferred to an agarose gel. The findings presented in Figures 5, 6, and 7 demonstrated the presence of bands with lengths of 565, 532, and 571 base pairs in the weed samples that were tested for sensitivity and resistance to herbicides, as well as in the samples from all three governorates (Babylon, Karbala, and Wasit). These band lengths correspond to the ACCase gene, which is the gene responsible for herbicide resistance. ACCase is the most important enzyme in the process of producing fatty acids, and it is one of the most typical targets of action for herbicides. It is a multifunctional enzyme that is readily

available in the body. It is required for the production of primary fatty acids, and it also plays a role in the biosynthesis of long-chain fatty acids [ 18 ].

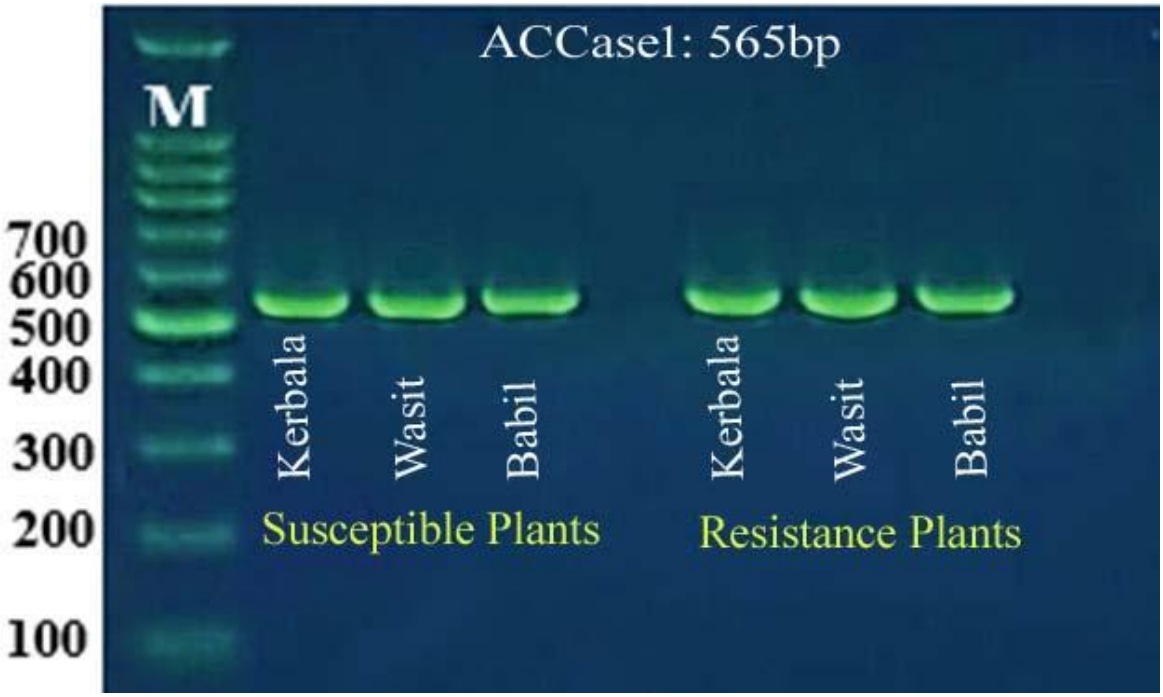



**Figure5:** *Electrophoresis of ACCase1 gene amplification results for sensitive and resistant Tobik and Axial plants in three Iraqi provinces after 45 minutes on 1% agarose gel at 95 volts.*

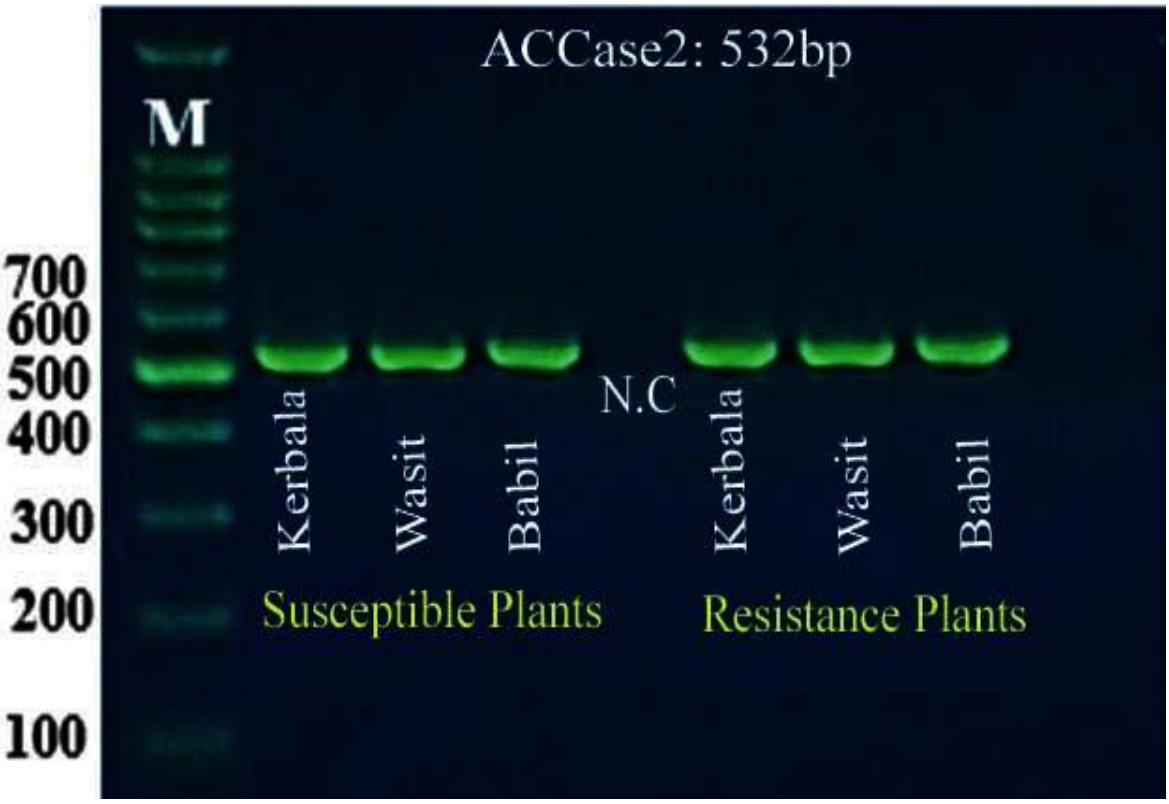



**Figure 6** *Electrophoresis of ACCase2 gene amplification of sensitive and resistant plants to Tobik and Axial in three Iraqi governorates after 45 minutes on 1% agarose gel at 95 V.*

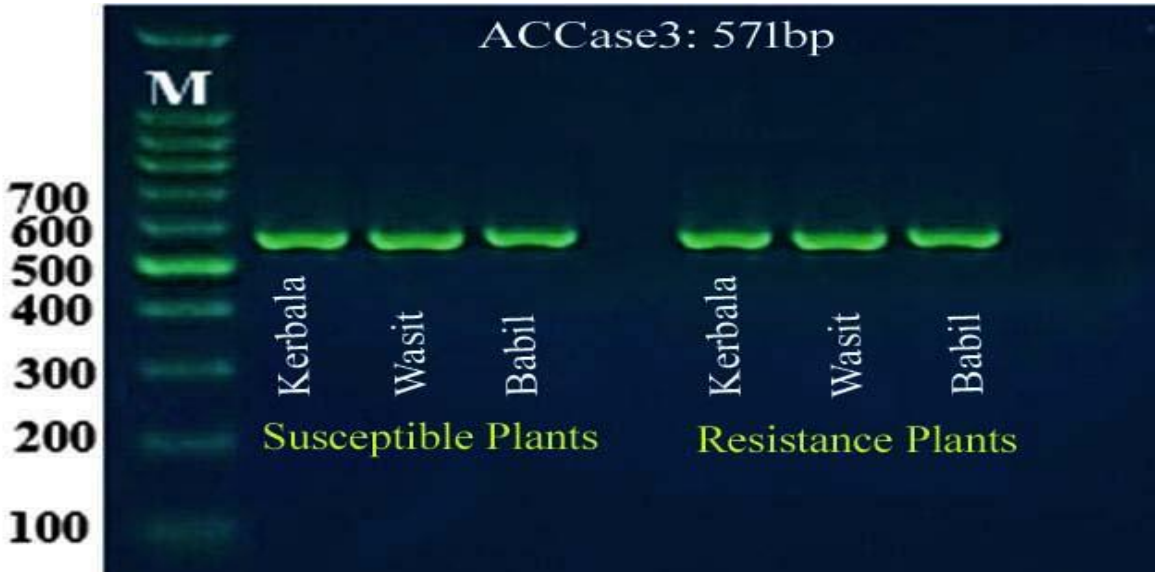



**Figure7:** *Electrophoresis of 3ACCase gene amplification data for plants sensitive and resistant to Tobik and Axial in three Iraqi provinces after 45 minutes on 1% agarose gel at 95 volts.*

***Genetic match and tree depending on the sequence of the ALS gene.***

Wasit, which encodes the enzyme Acetolactate synthase responsible for the biosynthesis of a large number of amino acids, the most important of which are valine, leucine, and isoleucine in wheat weed (Lolium rigidum), as it is concentrated in wheat weed (Lolium rigidum), in order to determine the amount of genetic match in the ALS gene between weed samples in three Iraqi governorates: Babylon and Karbala. This was done in order to determine the This match was studied at the level of nucleotides between the weed samples of the three provinces and for sensitive and herbicide-resistant plants, as well as comparing them with sequences recorded in the National Center for Biotechnology Information (NCBI) for two plants, one sensitive to herbicides (MH165308.1) and the other resistant to herbicides. Since Pallas and Chevalier weed pesticides inhibited the action of this enzyme and thus caused the death of weed The findings presented in Table 1 demonstrated that the weed samples that were sensitive to herbicides in the three governorates matched the nucleotide sequence of the ALS gene among themselves. In addition, they matched the global sensitive cultivar by 100% as a result of the absence of any change in the sequence .Given these findings, it is possible that the weeds that are scattered throughout the world are descended from a single source [ 19 ]The results also showed that the match rate between the resistant plants in Wasit and Karbala governorates reached 100%, and the match rate between the resistant plants of Babylon and the resistant global plants reached 100%; however, they matched 99.05% with the resistant plants in Wasit and Karbala as a result of a change in the number of nucleotides in the ALS gene sequence. This was due to the fact that the resistant plants in Babylon had a different number of nucleotides than the resistant plants in Was. According to the results of, which showed that 91% of the weed was resistant to the action of the herbicide by inhibiting the ALS enzyme, these changes can be repeated worldwide, and new mutations can also appear, as happened in the governorates of Karbala and Wasit. This is what Confirmed by the results of Appendix 1, which showed that 91% of the weed was resistant to the action of the herbicide. The results of this gene were the same in the weed (Lolium temulentum) as they were in the weed (Lolium rigidum), with the exception of the existence of one silent mutation in the codon number 150 GCT that encodes for acid. The codon that encodes for the amino acid Ala was changed in the weed from Wasit Governorate to the codon GCC, which encodes for the same amino acid as shown in Figure (8).

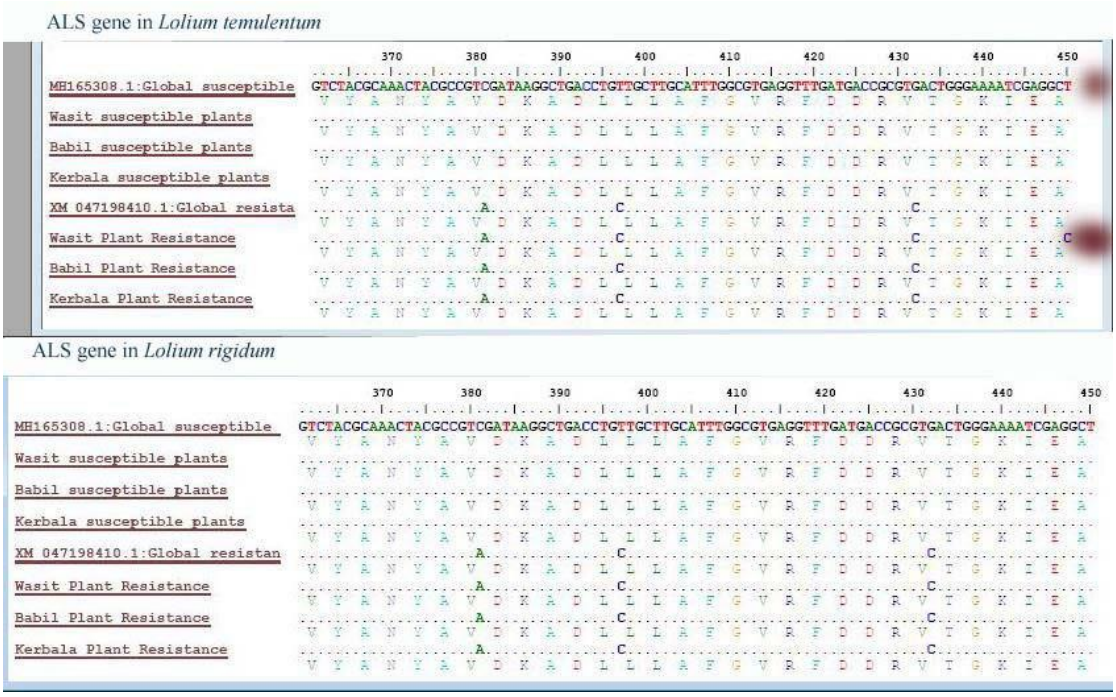



**Figure 8** *The difference in sequences between weed*

**Table 7:** *The amount of match between plants resistant and sensitive to herbicides with the ALS gene.*

| **Babylon is resistant** | | **Karbala is resistant** | | **XM_047198410.1** | | **sensitive medium** | | **Babel is sensitive** | | **Karbala is sensitive** | | **MH165308.1** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | |
| | | | | | | | | | | | | | | MH165308.1 |
| | | | | | | | | | | | | **0.0** | **100** | Karbala is sensitive |
| | | | | | | | | | | **0.0** | **100** | **0.0** | **100** | Babel is sensitive |
| | | | | | | | | **0.0** | **100** | **0.0** | **100** | **0.0** | **100** | sensitive medium |
| | | | | | | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | XM_047198410.1 |
| | | | | **0.0** | **100** | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | Karbala is resistant |
| | | **0.0** | **100** | **0.0** | **100** | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | **0.0** | **98.04** | Babylon is resistant |
| **0.0** | **99.84** | **0.0** | **99.84** | **0.0** | **99.84** | **0.0** | **97.88** | **0.0** | **97.88** | **0.0** | **97.88** | **0.0** | **97.88** | Resistant mediator |

Following the information presented above, a genetic tree was constructed (shown in Figure 8) based on the degree of similarity between the nucleotides of plants that are sensitive to the action of herbicides and plants that are resistant to the action of herbicides. Nitrogenous,

whereas group B included plants that were resistant to the action of the pesticide. It was found that the resistant plants of Wasit province had genetically diverged from the resistant plants of the provinces of Babylon and Karbala in addition to the global resistant variety, as a result of the presence of mutations that were specific to them. This was the case because of the presence of mutations that were unique to them.

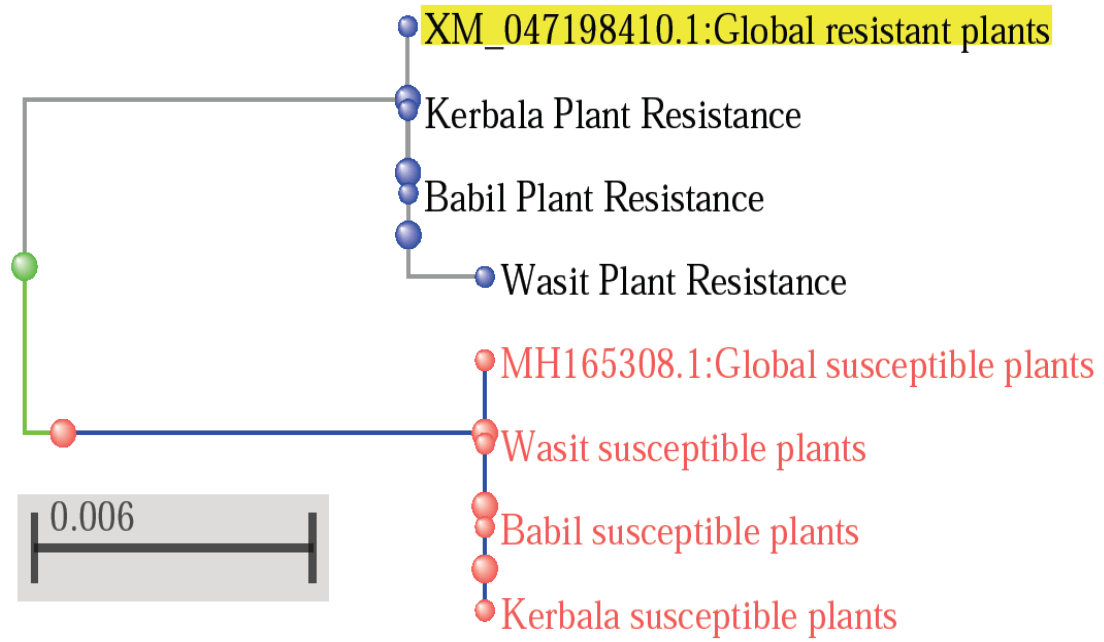



**Figure 9:** *Genetic tree based on the nucleotide sequence of the ALS gene*

## Mutations in the ALS gene and their effects

In comparison to plants that are sensitive to the activity of herbicides, plants that are resistant to its effects have a number of changes in the nucleotide sequence of the ALS gene, as shown by the findings in Table 7. and CGC, GTC, TTG, GTG, ATT, GAT, ACT, TTG, GGC, GGT, CGT, GCG, and ACT to the codons CCG, GGC, CCG, CTC, CCT, CTA, GGT, GCG, ACT, CTC, CGT, GTA, CTG, GTC, ATC, GAC, and ACC, respectively; however, they continued to encode (for the same amino acids, Pro In addition, the results of the table demonstrated the presence of a number of different mutations. Missense mutation was found in the sequences of plants from the governorates of Karbala, Wasit, and Babel. This mutation is comparable to the mutation that was discovered in resistant plants that were recorded worldwide. This mutation was caused by the substitution of nucleotides with others in codons 195 and 200. Specifically, the nucleotides AGC and AGT, which encode the amino acid Ser, were changed to ACC and GGT, which encode the amino acids Thr and Gly. These mutations The results also demonstrated the presence of two missense mutations in codons 175 and 266 of the sequence of mediator plants that were resistant to the action of herbicides. These mutations involved the substitution of codons GTC and CGC that encode for the amino acids Val and Arg with codons GGC and TGC that encode for the amino acids Gly and Cys sequentially. These findings are consistent with what [20] .presented in their findings.

**Table 8 a:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ALS gene*

| 47 | **Amino Acid** | **Pro** | **Pro** | **Pro** | **Pro** | **Pro Silent mutation** | **Pro Silent mutation** | **Pro Silent mutation** | **Pro Silent mutation** |
|---|---|---|---|---|---|---|---|---|---|
| | Codon | CCT | CCT | CCT | CCT | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio |
| 17 | Amino Acid | Leu | Leu | Leu | Leu | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation |
| | Codon | CTG | CTG | CTG | CTG | CTC Substitution - Transitio | CTC Substitution - Transitio | CTC Substitution - Transitio | CTC Substitution - Transitio |
| 15 | Amino Acid | Pro | Pro | Pro | Pro | Pro Silent mutation | Pro Silent mutation | Pro Silent mutation | Pro Silent mutation |
| | Codon | CCC | CCC | CCC | CCC | CCG Substitution - Transitio | CCG Substitution - Transitio | CCG Substitution - Transitio | CCG Substitution - Transitio |
| 14 | Amino Acid | Gly | Gly | Gly | Gly | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation |
| | Codon | GGG | GGG | GGG | GGG | GGC Substitution - Transitio | GGC Substitution - Transitio | GGC Substitution - Transitio | GGC Substitution - Transitio |
| 13 | Amino Acid | Pro | Pro | Pro | Pro | Pro Silent mutation | Pro Silent mutation | Pro Silent mutation | Pro Silent mutation |
| | Codon | CCA | CCA | CCA | CCA | CCG Substitution - Transitio | CCG Substitution - Transitions | CCG Substitution - Transitio | CCG Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 8b:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ALS gene*

| 110 | **Amino Acid** | **Leu** | **Leu** | **Leu** | **Leu** | **Leu Silent mutation** | **Leu Silent mutation** | **Leu Silent mutation** | **Leu Silent mutation** |
|---|---|---|---|---|---|---|---|---|---|
| | Codon | CTG | CTG | CTG | CTG | CTC Substitution - Transitio | CTC Substitution - Transitio | CTC Substitution - Transitio | CTC Substitution - Transitio |
| 90 | Amino Acid | The | The | The | The | The Silent mutation | The Silent mutation | The Silent mutation | The Silent mutation |
| | Codon | ACC | ACC | ACC | ACC | ACT Substitution - Transitio | ACT Substitution - Transitio | ACT Substitution - Transitio | ACT Substitution - Transitio |
| 78 | Amino Acid | Ala | Ala | Ala | Ala | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation |
| | Codon | GCA | GCA | GCA | GCA | GCG Substitution - Transitio | GCG Substitution - Transitio | GCG Substitution - Transitio | GCG Substitution - Transitio |
| 62 | Amino Acid | Gly | Gly | Gly | Gly | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation |
| | Codon | GGC | GGC | GGC | GGC | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio |
| 55 | Amino Acid | Leu | Leu | Leu | Leu | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation |
| | Codon | CTC | CTC | CTC | CTC | CTA Substitution - Transitio | CTA Substitution - Transitio | CTA Substitution - Transitio | CTA Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 8c:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with ALS gene.*

| | Amino Acid | Arg | Arg | Arg | Arg | Arg Silent mutation | Arg Silent mutation | Arg Silent mutation | Arg Silent mutation |
|---|---|---|---|---|---|---|---|---|---|
| **162** | Codon | ATT | ATT | ATT | ATT | ATC Substitution - Transitio | ATC Substitution - Transitio | ATC Substitution - Transitio | ATC Substitution - Transitio |
| 144 | Amino Acid | Val | Val | Val | Val | Val Silent mutation | Val Silent mutation | Val Silent mutation | Val Silent mutation |
| | Codon | GTG | GTG | GTG | GTG | GTC Substitution - Transitio | GTC Substitution - Transitio | GTC Substitution - Transitio | GTC Substitution - Transitio |
| 133 | Amino Acid | Leu | Leu | Leu | Leu | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation |
| | Codon | TTG | TTG | TTG | TTG | CTG Substitution - Transitio | CTG Substitution - Transitio | CTG Substitution - Transitio | CTG Substitution - Transitio |
| 127 | Amino Acid | Val | Val | Val | Val | Val Silent mutation | Val Silent mutation | Val Silent mutation | Val Silent mutation |
| | Codon | GTC | GTC | GTC | GTC | GTA Substitution - Transitio | GTA Substitution - Transitio | GTA Substitution - Transitio | GTA Substitution - Transitio |
| 113 | Amino Acid | Arg | Arg | Arg | Arg | Arg Silent mutation | Arg Silent mutation | Arg Silent mutation | Arg Silent mutation |
| | Codon | CGC | CGC | CGC | CGC | CGT Substitution - Transitio | CGT Substitution - Transitio | CGT Substitution - Transitio | CGT Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 8 d:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ALS gene*

| 223 | Amino Acid | The | The | The | The | The Silent mutation | The Silent mutation | The Silent mutation | The Silent mutation |
|---|---|---|---|---|---|---|---|---|---|
| | Codon | ACT | ACT | ACT | ACT | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio |
| 202 | Amino Acid | Asb | Asb | Asb | Asb | Asb Silent mutation | Asb Silent mutation | Asb Silent mutation | Asb Silent mutation |
| | Codon | GAT | GAT | GAT | GAT | GAC Substitution - Transitio | GAC Substitution - Transitio | GAC Substitution - Transitio | GAC Substitution - Transitio |
| 200 | Amino Acid | Ser | Ser | Ser | Ser | Gly Missense mutation | Gly Missense mutation | Gly Missense mutation | Gly Missense mutation |
| | Codon | AGT | AGT | AGT | AGT | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio |
| 195 | Amino Acid | Ser | Ser | Ser | Ser | Thr Missense mutation | Thr Missense mutation | Thr Missense mutation | Thr Missense mutation |
| | Codon | AGC | AGC | AGC | AGC | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio |
| 175 | Amino Acid | Val | Val | Val | Val | Val | Val | Val | Gly Missense mutation |
| | Codon | GTC | GTC | GTC | GTC | GTC | GTC | GTC | GGC Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 8E:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with ALS gene*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **380** | **Amino Acid** | **Arg** | **Arg** | **Arg** | **Arg** | **Arg Silent mutation** | **Arg Silent mutation** | **Arg Silent mutation** | **Arg Silent mutation** |
| | Codon | CGT | CGT | CGT | CGT | CGG Substitution - Transitio | CGG Substitution - Transitio | CGG Substitution - Transitio | CGG Substitution - Transitio |
| | Amino Acid | Gly | Gly | Gly | Gly | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation |
| 295 | Codon | GGT | GGT | GGT | GGT | GGG Substitution - Transitio | GGG Substitution - Transitio | GGG Substitution - Transitio | GGG Substitution - Transitio |
| | Amino Acid | Gly | Gly | Gly | Gly | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation | Gly Silent mutation |
| 288 | Codon | GGC | GGC | GGC | GGC | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio | GGT Substitution - Transitio |
| | Amino Acid | Leu | Leu | Leu | Leu | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation | Leu Silent mutation |
| 283 | Codon | TTG | TTG | TTG | TTG | CTG Substitution - Transitio | CTG Substitution - Transitio | CTG Substitution - Transitio | CTG Substitution - Transitio |
| | Amino Acid | Arg | Arg | Arg | Arg | Arg | Arg | Arg | Cys Missense mutation |
| 266 | Codon | CGC | CGC | CGC | CGC | CGC | CGC | CGC | TGC Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 8F:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with ALS gene*

| **283** | **Amino Acid** | **Thr** | **Thr** | **Thr** | **Thr** | **Thr Silent mutation mutation** | **Thr Silent mutation** | **Thr Silent mutation** | **Thr Silent mutation** |
|---|---|---|---|---|---|---|---|---|---|
| | Codon | ACT | ACT | ACT | ACT | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio | ACC Substitution - Transitio |
| | Amino Acid | Ala | Ala | Ala | Ala | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation |
| 266 | Codon | CCG | CCG | CCG | CCG | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

***Genetic match and genetic tree depending on the sequence of the ACCase gene.***

As the work of herbicides in Tobik and Axial is focused on inhibiting the action of this enzyme and thus the death of the weed [ 21 ], so this correspondence was studied in order to determine the amount of genetic match in the ACCase gene between weed samples in three Iraqi governorates (Babylon, Karbala, and Wasit). This was done in order to determine the amount of genetic match in the ACCase gene between (DQ184646.1). The findings presented in Table 13 demonstrated that the weed samples that were sensitive to the action of herbicides in the three governorates shared a nucleotide sequence of the ACCase gene among themselves. Furthermore, these weed samples matched the nucleotide sequence of the globally sensitive cultivar by a factor of 100% because there was no change in the sequence .This pairing may lend support to the notion that the marijuana is. The spread in the world is the result of a single point of origin [ 22 ] .and as a result, he is able to work and eliminate them when manufacturing herbicides. However, since this sensitive weed accounts for 12% of the total weed studied, it is not possible to manufacture single herbicides that will eliminate the spread weed, and this is what has caused the problem. It was also shown by the results of the table when studying the correspondence between plants resistant to the action of herbicides and for the three governorates in addition to comparing them with global resistant plants, as the percentage of correspondence between resistant plants in the provinces of Wasit and Karbala amounted to 100%, as well as the percentage of correspondence between resistant plants of Babylon and global resistant plants reached 100%, but they did not match.

This was shown by the fact that the percentage of correspondence between resistant plants in the province of Babylon and global resistant plants. The familiarity with globally resistant plants indicates that these changes can recur worldwide, as well as new mutations can appear, as it happened in the governorates of Karbala and Wasit, which indicates that the use of single herbicides in different regions and countries may not be feasible, and this is confirmed by the results of the which showed that 88% of the weeds are resistant to the action of herbicides ACCase.

**Table 9** *displays the percentage of matches between herbicide-resistant plants and herbicide-sensitive plants that have the ACCase gene.*

| **Babylon is resistant** | | **Karbala is resistant** | | **XM_047198410.1** | | **sensitive medium** | | **Babel is sensitive** | | **Karbala is sensitive** | | **DQ184647.1** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | **E Value** | **Ident %** | |
| | | | | | | | | | | | | | | DQ184647.1 |
| | | | | | | | | | | | | 0.0 | 100 | Karbala is sensitive |
| | | | | | | | | | | 0.0 | 100 | 0.0 | 100 | Babel is sensitive |
| | | | | | | | | 0.0 | 100 | 0.0 | 100 | 0.0 | 100 | sensitive medium |
| | | | | | | 0.0 | 99.16 | 0.0 | 99.16 | 0.0 | 99.16 | 0.0 | 99.16 | XM_047198410.1 |
| | | | | 0.0 | 99.05 | 0.0 | 98.42 | 0.0 | 98.42 | 0.0 | 98.42 | 0.0 | 98.42 | Karbala is resistant |
| | | 0.0 | 99.05 | 0.0 | 100.00 | 0.0 | 99.16 | 0.0 | 99.16 | 0.0 | 99.16 | 0.0 | 99.16 | Babylon is resistant |
| 0.0 | 99.05 | 0.0 | 100.00 | 0.0 | 99.05 | 0.0 | 98.42 | 0.0 | 98.42 | 0.0 | 98.42 | 0.0 | 98.42 | Resistant mediator |

Through the foregoing, a genetic tree was drawn (Fig. 11) based on the amount of match between the nucleotides of plants that are sensitive and resistant to the action of herbicides. As for group B, it included two sub-groups, group B1 contained resistant plants from Wasit and Karbala governorates, while group B2 included resistant plants from Babel governorate with globally registered resistant plants.

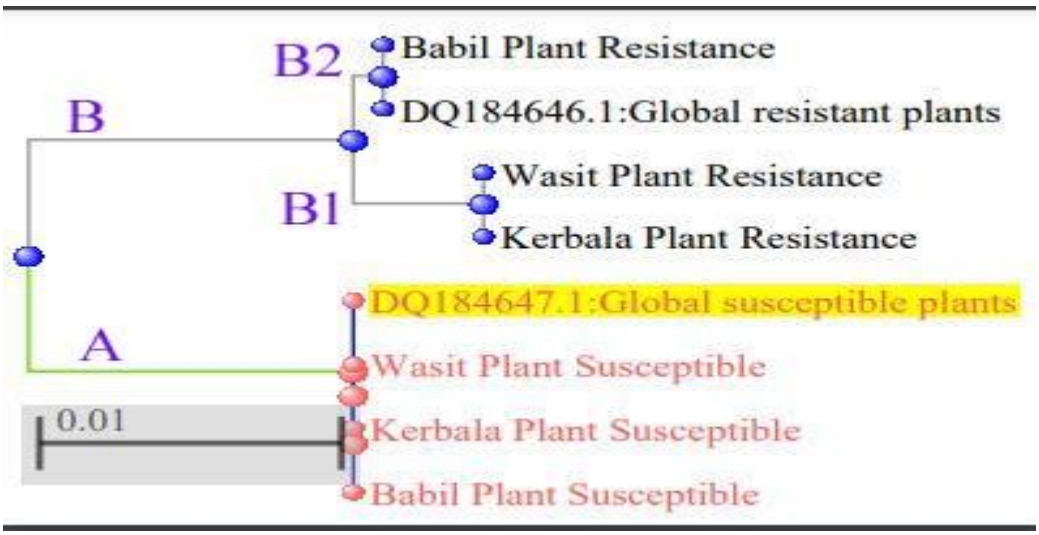



**Figure10:** *Genetic tree between plants sensitive and resistant to herbicides based on ACCase gene in three Iraqi provinces.*

***Mutations in the ACCase gene and their effects.***

In comparison to plants that are sensitive to the activity of herbicides, plants that are resistant to its effects have a number of changes in the nucleotide sequence of the ACCase gene, as shown by the findings in Table 13 As three silent mutations appeared in the plants of Karbala, Wasit, and Babel governorates, which are similar to the mutation found in resistant plants recorded worldwide, by substituting other nucleotides in codons 15, 146, and 307 from GCG, TCT, and ATA to GCA, TCC, and ATC, however, it continued coding for the same amino acids. This was due to the fact that the mutation was similar to the mutation found in resistant plants recorded worldwide (Ala, Ser, and Ile, respectively). The results of the table also showed the presence of a number of missense mutations in the sequences of plants in the governorates of Karbala, Wasit, and Babel. These mutations are comparable to the mutation that was found in resistant plants recorded worldwide, and they were caused by the substitution of other nucleotides in codons 25, 29, 33, 167, and 209 of CGA and GAA. And AAT, TTG, and ACA, which encode the amino acids Arg, Glu, Asn, and Leu, to CCA, GCA, CAT, ATG, and ATA, which encode Pro, Ala, His, Met, and Ile. Also, CCA, GCA, and ATA encode Pro, Ala, His, Met, and Ile. The results showed the presence of a missense mutation in codon 161 of the Wasit and Karbala sequence plants that are resistant to the action of herbicides. This mutation was caused by the substitution of two nitrogenous bases from the codon AAG, which encodes to Lys, to AGA, which encodes to Arg. Mutations recorded globally [ 23 ].and the results also showed the presence of a missense mutation in codon 161 of the Wasit and Karbala sequence plants. This mutation caused a change in the reading region after that in codons 164 and 165 to change the coding to the amino acid, and it was noted that there were two frame shift mutations in herbicide-resistant plants in the governorates of Karbala and Wasit. One of these mutations was an insertion base A, and it caused codon 136 AGC to encode the amino acid Ser. This mutation also caused a change in the reading region after that in codons 164 and 165 to change the Ala and Asp were substituted one after the other before the change was halted by a deletion mutation in codon 165, which was necessary to fix the reading of the frame. These findings validate the previous assertions made by [ 24 ].

**Table 10a:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ACCase gene*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Amino Acid** | **Ser** | **Ser** | **Ser** | **Ser** | **Ser Silent mutation** | **Ser Silent mutation** | **Ser Silent mutation** | **Ser Silent mutation** |
| **146** | Codon | TCT | TCT | TCT | TCT | TCC Substitution - Transitio | TCC Substitution - Transitio | TCC Substitution - Transitio | TCC Substitution - Transitio |
| | Amino Acid | Asn | Asn | Asn | Asn | His Missense mutation | His Missense mutation | His Missense mutation | His Missense mutation |
| 33 | Codon | ATT | ATT | ATT | ATT | CTT Substitution - Transitio | CTT Substitution - Transitio | CTT Substitution - Transitio | CTT Substitution - Transitio |
| | Amino Acid | Gly | Gly | Gly | Gly | Ala Missense mutation | Ala Missense mutation | Ala Missense mutation | Ala Missense mutation |
| 29 | Codon | GAA | GAA | GAA | GAA | GCA Substitution - Transitio | GCA Substitution - Transitio | GCA Substitution - Transitio | GCA Substitution - Transitio |
| | Amino Acid | Arg | Arg | Arg | Arg | Pro Missense mutation | Pro Missense mutation | Pro Missense mutation | Pro Missense mutation |
| 25 | Codon | CGA | CGA | CGA | CGA | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio | CCA Substitution - Transitio |
| | Amino Acid | Ala | Ala | Ala | Ala | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation | Ala Silent mutation |
| 15 | Codon | GCG | GCG | GCG | GCG | GCA Substitution - Transitio | GCA Substitution - Transitio | GCA Substitution - Transitio | GCA Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

**Table 10b:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ACCase gene*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **166** | **Amino Acid** | **Met** | **Met** | **Met** | **Met** | **Met** | **Met Silent mutation** | **Met Silent mutation** |
| | Codon | ATG | ATG | ATG | ATG | ATG | A-TG= ATG Deletion | A-TG= ATG Deletion |
| 165 | Amino Acid | Ala | Ala | Ala | Ala | Ala | Asp Missense mutation | Ala As Missense mutation p |
| | Codon | GCA | GCA | GCA | GCA | GCA | GAT Substitution - Transitio | GCA GAT Substitution - Transitio |
| 164 | Amino Acid | Gly | Gly | Gly | Gly | Gly | Ala Missense mutation | Gly Ala Missense mutation |
| | Codon | CAG | CAG | CAG | CAG | CAG | GCA | CAG GCA |
| 163 | Amino Acid | Ala | Ala | Ala | Ala | Ala | Ser Missense mutation | Ala Ser Missense mutation |
| | Codon | -GCtoGCG | -GCtoGCG | -GCtoGCG | -GCtoGCG | -GCtoGCG | AGC Instion | -GCtoGCG AGC Instion |
| 161 | Amino Acid | Lys | Lys | Lys | Lys | Lys | Arg Missense mutation | Lys Arg Missense mutation |
| | Codon | AAG | AAG | AAG | AAG | AAG | AGA Substitution - Transitio | AAG AGA Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant Wasit resistant |

**Table 10c:** *Mutations and their effects on amino acids in plants sensitive and resistant to herbicides with the ACCase gene*

| 313 | **Amino Acid** | **Gln** | **Gln** | **Gln** | **Gln** | **Gln** | **Ser Silent mutation** | **Gln** | **Ser Silent mutation** |
|---|---|---|---|---|---|---|---|---|---|
| | Codon | CAG | CAG | CAG | CAG | CAG | AGT Substitution – Transitio and Transition | CAG | AGT Substitution and – Transitio Transition |
| 307 | Amino Acid | Ile | Ile | Ile | Ile | Ile Silent mutation | Ile Silent mutation | Ile Silent mutation | Ile Silent mutation |
| | Codon | ATA | ATA | ATA | ATA | ATC Substitution - Transitio | ATC Substitution - Transitio | ATC Substitution - Transitio | ATC Substitution - Transitio |
| 209 | Amino Acid | The | The | The | The | Ile Missense mutation | Ile Missense mutation | Ile Missense mutation | Ile Missense mutation |
| | Codon | ACA | ACA | ACA | ACA | ATA Substitution - Transitio | ATA Substitution - Transitio | ATA Substitution - Transitio | ATA Substitution - Transitio |
| 167 | Amino Acid | Leu | Leu | Leu | Leu | Met Missense mutation | Met Missense mutation | Met Missense mutation | Met Missense mutation |
| | Codon | TTG | TTG | TTG | TTG | ATG Substitution - Transitio | ATG Substitution - Transitio | ATG Substitution - Transitio | ATG Substitution - Transitio |
| position | | DQ184647.1 | Karbala is sensitive | Babel sensitive | Wasit sensitive | DQ184646.1 | Karbala resistant | Babel resistant | Wasit resistant |

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