Genetic characterization of the *acetohydroxyacid synthase* (*AHAS*) gene responsible for imidazolinone tolerance in chickpea (C*icer arietinum* L.).

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Abstract

Weed control in chickpea (*Cicer arietinum* L.) is challenging because of poor crop competition ability and limited herbicide options. Development of chickpea varieties with resistance to different herbicide modes of action would be desirable. Resistance to imidazolinone (IMI) herbicides in chickpea has been previously identified, but the genetic inheritance and the mechanism were unknown. In many plant species, IMI resistance is caused by point mutation(s) in the *acetohydroxyacid synthase* (*AHAS*) gene resulting in an amino acid substitution. This changes the enzyme configuration at the herbicide binding site, preventing the herbicide attachment to the molecule. The main research objective was to genetically characterize chickpea resistance to imidazolinone herbicides. Two homologous *AHAS* genes, namely *AHAS1* and *AHAS2* sharing 80% similarity were identified in the chickpea genome. A point mutation in *AHAS1* at cytosine 675→ thymine 675 resulting in an amino acid substitution from alanine 205 to valine 205 confers the resistance to imidazolinone in chickpea. A KASP marker targeting the point mutation was developed and effectively predicted the herbicide response in the RIL population. This same population was used in molecular mapping where the major locus for herbicide resistance was mapped to chromosome 5. Segregation analysis demonstrated that the resistance is inherited as a single gene in a semi-dominant fashion. To study the synteny of *AHAS* across plant species, lentil (*Lens culinaris*) *AHAS1* was sequenced. The same mutation that confers the resistance to imidazolinone in chickpea was also found in lentil. Phylogenetic analysis indicated independent clustering of *AHAS1* and *AHAS2* across pulse species. *In vivo* and *in vitro* AHAS enzyme activity analysis showed inhibition of AHAS activity in the susceptible genotype CDC Frontier over time and with the increasing imidazolinone concentrations. In contrast, the resistant genotype CDC Cory did not show AHAS inhibition under the same treatments. In summary, the simple genetic inheritance and the availability of KASP marker could aid in the development of chickpea varieties with resistance to imidazolinone herbicide.

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Table of Contents

Permission to Use i
Abstract ii
Acknowledgments iii
Table of Contents iv
List of Tables vi
List of Figures vi
List of Appendices vii
List of Abbreviations Used viii
Introduction 1
Research Hypotheses and Objectives 3
1. Review of Literature 4
1.1. Chickpea 4
1.1.1. Chickpea Biology 4
1.1.2. *Cicer* Classification and Origin 5
1.1.3. Agricultural Production 5
1.1.4. Weed Management 7
1.2. Imidazolinone Herbicides 8
1.2.1. Background 8
1.2.2. AHAS/ALS Enzyme 10
1.2.3. Mode of Action 11
1.2.4. Agronomic Advantages and Disadvantages 12
1.2.5. Cross Resistance of AHAS Inhibiting Herbicides 13
1.3. Developing Imidazolinone Herbicide Resistance 13
1.3.1. Plant Breeding Techniques 13
1.3.2. Inheritance of Resistance 14
1.3.3. Previous Genetic Characterization (*AHAS* sequencing) 14
2. Materials and Methods 17
2.1. Component 1: Sequencing *acetohydroxyacid synthase* (*AHAS*) gene in chickpea 17
2.1.1. Plant Material 17

2.1.2. PCR and Sequencing Preparation 18
2.1.3. KASP SNP genotyping preparation 21
2.2. Component 2: Synteny of *AHAS* Gene 23
2.2.1. Sequencing Lentil *AHAS* Gene 23
2.2.2. Cluster Analysis 23
2.3. Component 3: Inheritance and Molecular Mapping of *AHAS1* Gene 25
2.3.1. Plant Material 25
2.3.2. Screening RILs for Herbicide Resistance 25
2.3.3. SNP Illumina GoldenGate® Assay 26
2.3.4. Linkage Analysis 27
2.4. Component 4: AHAS Enzyme Activity 28
3. Results 30
3.1. Component 1: Sequencing *Acetohydroxyacid Synthase* (*AHAS*) Gene 30
3.1.1. Sequence Analysis 30
3.1.2. KASP SNP Genotyping 32
3.2. Component 2: Synteny of *AHAS* Gene 33
3.2.1. Lentil *AHAS* Sequencing 33
3.2.2. Cluster Analysis 34
3.3. Component 3: Molecular Mapping of *AHAS1* Gene 36
3.3.1. Molecular Mapping 36
3.3.2. Inheritance 38
3.4. AHAS Enzyme Activity 39
4. Discussion 43
Summary and Conclusions 50
References 52
Appendices 62

List of Tables

Table 1.1. 2011 World chickpea production by country (FAOSTAT 2013). 6
Table 1.2. Canadian chickpea production statistics (Statistics Canada, 2012).....6
Table 1.3: Summary of weed control characteristics and agronomic use of different imidazolinone herbicides registered for use on Saskatchewan field crops (Saskatchewan Ministry of Agriculture 2013) 9
Table 2.1. Characteristics of chickpea varieties used for *AHAS* genetic characterization 18
Table 3.1. NCBI BLAST® query results of searching *Cicer arietinum AHAS1* sequence.....34
Table 3.2: Comparison of CDC512-51 x ICCX860047-9 RIL chickpea genetic map with ICCV96029xCDC Frontier consensus map 37
Table 3.3. Chi square statistics for CDC512-51 x ICCX 860047-9 population segregating for IMI herbicide resistance 39

List of Figures

Figure 1.1 Biosynthesis of branched chain amino acids (Zhou *et al.* 2007) 11

Figure 2.1 Diagrammatic representation of A) KASP reaction components and B) final signal generation step of KASP SNP genotyping. Genomic DNA is used in allele specific PCR resulting in bi-allelic SNP calling through FAM^{TM} and HEX^{TM} fluorescently labeled oligonucleotides. The point mutation site that confers to IMI resistance in chickpea is indicated in yellow 22

Figure 2.2 Chickpea check varieties in response to IMI herbicide at 7 days after treatment with Solo® (imazamox) at a rate of 20.22 g a.i. ha^{-1} (11.7 g. Solo®/acre), treatment was applied at plant age between 17 – 21 days after seeding A) left, CDC Cory IMI resistant B) right, CDC Corinne IMI susceptible. 26

Figure 3.1: Nucleotide and amino acid sequence alignments, susceptible genotype highlighted in yellow, resistant genotype highlighted in blue A) nucleotide alignment across seven chickpea (*Cicer arietinum*) genotypes, B) amino acid alignment across seven chickpea (*Cicer arietinum*) genotypes, C) amino acid alignment of chickpea and model plant *AHAS1* sequences. (Alignment based off of *Arabidopsis thaliana* NCBI reference NM_114714.2).....31

Figure 3.2: KASP SNP genotyping allelic discrimination plot of $F_{7:8}$ CDC 512-51 x ICCX 860047-1 population segregating for resistance to IMI herbicide. (Alignment based off of *Arabidopsis thaliana* NCBI reference NM_114714.2) A = parental type CDC 512-51 (IMI susceptible; red) and B = parental type ICCX860047-1 (IMI resistant; blue) 32

Figure 3.3. *AHAS1* Nucleotide and Amino acid sequence alignment of chickpea and lentil. (Alignment based on *Arabidopsis thaliana* NCBI reference NM_114714.2).....33

Figure 3.4. Phylogenetic tree of *AHAS1* and *AHAS2* in multiple plant species, using Neighbour-Joining method and bootstrap confidence levels (bootstrap replications = 500). Bootstrap confidence intervals are placed at each node and are expressed as a percentage. 35

Figure 3.5: RIL parents' response to 1x rate of imidazolinone herbicide of Solo® (imazamox) at a rate of 20.22 g a.i. ha^{-1} (11.7 g. Solo®/acre), A) ICCX860047-9 IMI resistant, B) CDC 512-51 IMI susceptible 37

Figure 3.6: Comparison of *Cicer arietinum* Chromosome 5 of CDC512-51 x ICCX860047-9 RIL population segregating for IMI resistance to ICCV96029 x CDC Frontier consensus map (genetic distance in Kosambi cM). 38

Figure 3.7. A) *In vitro* colorimetric assay for AHAS enzyme activity with increasing IMI concentration for CDC Frontier (IMI susceptible) and CDC Cory (IMI resistant), B) graphical representation of colorimetric assay (absorbance values converted to acetolactate concentration using a standard curve). 40

Figure 3.8. A) *In vivo* colorimetric assay for AHAS enzyme activity over time after treatment with IMI for CDC Frontier (IMI susceptible) and CDC Cory (IMI resistant), B) graphical representation of colorimetric assay (absorbance values converted to acetolactate concentration using a standard curve). 41

Figure 4.1.Common mutation sites in the *AHAS* gene that cause resistance to Group 2 herbicides across various plant species (amino acid numbering in arrows based on the *Arabidopsis thaliana* NCBI reference NM_114714.2) (Tan *et al.* 2005). Pulse species holding the specific mutation site causing Group 2 resistance is indicated above the mutation site arrow. 44

List of Appendices

Appendix 1: *Cicer arietinum* L. IMI Susceptible Consensus AHAS Sequences.....62
Appendix 2: *Cicer arietinum* L. IMI Resistant Consensus AHAS sequences.....64
Appendix 3: *Lens culinaris* Consensus AHAS sequences.....67
Appendix 4: Chickpea (*Cicer arietinum L.*) AHAS1 nucleotide sequence alignment of 70
IMI-S and IMI-R genotypes
Appendix 5: AHAS1 amino acid multiple alignment of *Arabidopsis thaliana, Medicago spp, Cicer arietinum* and *Lens culinaris*.....76
Appendix 6: Chromosome Genetic Maps.....79
Appendix 7: IMI Herbicide ratings and KASP SNP genotyping results.....81
Appendix 8: AHAS Enzyme Activity Additional Data.....89

List of Abbreviations Used

AHAS	Acetohydroxyacid Synthase
ALS	Acetolactate Synthase
BLAST	Basic Local Alignment Search Tool
CDC	Crop Development Centre
DAT	Days After Treatment
DNA	Deoxyribonucleic Acid
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IMI	Imidazolinone
KASP	KBioscience Allele-Specific PCR
LOD	Logarithm of Odds
ORF	Open Reading Frame
NCBI	National Center for Biotechnology
PCR	Polymerase Chain Reaction
PTB	Pyrimidinylthiobenzoates
qPCR	Quantitative Real-Time PCR
RIL	Recombinant Inbred Line
SCT	Sulfonylaminocarbonyltriazolinone
SU	Sulfonylureas
TAE	Tris-acetate
TP	Triazolopyrimidines
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

Introduction

Chickpea (*Cicer arietinum* L.*)* is a relatively new pulse crop in the Canadian Prairies. In Saskatchewan, chickpea is produced on Brown and Dark Brown soil zones of the south-western Saskatchewan (Baker *et al.* 1996; Padbury *et al.* 2002; Yadav 2007). Some agronomic issues that can decrease chickpea yield include *Ascochyta* blight disease, late maturity, frost damage and weed pressure. In general, herbicide Group 1 (*acetyl CoA carboxylase* inhibitors), and Group 2 (*acetohydroxyacid synthase* inhibitors) are commonly used for weed control in pulse crops in Saskatchewan (Saskatchewan Ministry of Agriculture 2013). Other herbicide groups are registered for minor use, but are specific to crop species or variety (Saskatchewan Ministry of Agriculture 2013). Chemical weed management options are limited in Saskatchewan chickpea production. Weed control in chickpea involves pre-seeding weed burn-offs (Glyphosate or 2-4, D) (Baker *et al.* 1996; McKay *et al.* 2002; Yadav 2007), pre-emergent (sulfentrazone) and post-emergent herbicide applications. However, chickpea crops can be damaged by soil residual activity of past herbicide applications which could result in yield reduction (Süzer and Büyük 2010; Saskatchewan Ministry of Agriculture 2013; Taran *et al.* 2013).

Post-emergence weed control in pulse crops can be challenging. Metribuzin (Group 5, photosynthetic inhibitor) is the only registered herbicide for post-emergence weed management in chickpea (Saskatchewan Ministry of Agriculture 2013). Metribuzin may cause leaf burn and stand thinning if applied late (Taran *et al.* 2013). Currently, imidazolinone herbicides are registered for use on non-pulse crops: barley, spring wheat, sunflower, oats, oilseed mustard, canola and alfalfa and pulse crops: lentil, field pea, soybean and dry bean (Saskatchewan Ministry of Agriculture 2013). Benefits of using imidazolinone herbicides include: low environmental impact, control of problem broadleaf weeds and low herbicide dose per acre

(Weed Science Society of America 2007; Saskatchewan Ministry of Agriculture 2013). Development of herbicide resistant chickpea varieties would provide more herbicide options for post emergence weed control and could reduce yield loss due to weed pressure (Kantar *et al.* 1999; Taran *et al.* 2010).

Resistance to the Group 2 herbicide imidazolinone has been identified in chickpea (Taran *et al.* 2010); however, the genetic inheritance and the mechanism leading to the resistance is unknown. Identifying the key gene and the mode of inheritance would help in understanding the herbicide resistance mechanism and could aid in the selection process to develop herbicide resistant varieties. In many plant species, resistance to Group 2 herbicides is the result of a point mutation in the *acetohydroxyacid synthase* gene causing amino acid substitution (Tan *et al.* 2005). Mutations may affect key herbicide binding sites, preventing Group 2 herbicides from binding and inhibiting acetohydroxyacid synthase enzyme activity (Muhitch *et al.* 1987; McCourt *et al.* 2006). Common AHAS amino acid substitutions causing Group 2 herbicide resistance include: Ala122, Pro197, Ala205 and Ser574 (Tan *et al.* 2005). Segregation studies suggested that Group 2 resistance is monogenic and has semi-dominant to dominant gene action in various plant species (Wright and Penner 1998; Pozniak and Hucl 2004; Oldach *et al.* 2008).

Single nucleotide polymorphism (SNP) markers as molecular tools are gaining attention because of automation potential, biallelic variation, high abundance in the genome and electrophoresis is not required (Rafalski 2002; Ganal *et al.* 2009). If Group 2 resistance is the result of a point mutation, a targeted SNP marker could be developed. This marker can then be used in marker assisted selection, increasing selection efficiency in developing resistant varieties (Mammadov *et al.* 2012). Additionally, SNP genotyping platforms like the Illumina GoldenGate® assay (Illumina, San Diego, CA, USA) can be used to quickly develop a molecular map and identify

the location of the gene responsible for the resistance to the herbicide in the plant genome (Rafalski 2002; Hiremath *et al.* 2012).

This study examined the genetic mechanism and developed an allele specific molecular marker that could be used in marker assisted selection for imidazolinone resistance in chickpea. The study also examined the synteny of the *AHAS* gene across *Medicago truncatula,* wild relatives of chickpea, field peas, lentil and other species.

Research Hypotheses and Objectives

The study was designed to test the following hypotheses: 1) Resistance to imidazolinone herbicides in chickpea is due to a point mutation in the *acetohydroxyacid synthase* (*AHAS*) gene; 2) Inheritance of resistance to imidazolinone herbicides in chickpea follows a single gene model, 3) SNP markers associated with the point mutation in *AHAS* gene can be used for selection of IMI resistant chickpea, 4) Point mutations in *AHAS* are conserved across imidazolinone resistant pulses, and 5) Imidazolinone inhibits the AHAS enzyme in IMI susceptible genotypes, but does not inhibit AHAS in the resistant genotypes.

The objectives of the research were 1) to sequence the *acetohydroxyacid synthase (AHAS)* gene in imidazolinone resistant chickpea and develop single nucleotide polymorphism (SNP) markers targeting the point mutation(s) causing imidazolinone resistance; 2) to examine the inheritance of the resistance using recombinant inbred lines (RILs) segregating for resistance to imidazolinone herbicide; 3) to test the usefulness of the SNP markers for selection of IMI resistant chickpea progeny; 4) to examine the synteny of the *AHAS* gene across pulse crops and other species; and 5) to examine AHAS enzyme activity in IMI susceptible and IMI resistant genotypes treated with the herbicide .

1. Review of Literature

1.1. Chickpea

1.1.1. Chickpea Biology

Chickpea (*Cicer arietinum* L.) is a diploid (2n = 2x = 16) legume crop grown on the Canadian Prairies. Kabuli and Desi are the two agricultural classes of chickpea, and each class differs in some physiological aspects (Moreno and Cubero 1978; Iruela *et al.* 2002). Kabuli seeds are round to ram head shaped and cream/white; while Desi seeds are small, angular in shape, with green, purple, brown or black thickened seed coat (Moreno and Cubero 1978; Pundir *et al.* 1985; Iruela *et al.* 2002). Chickpea will germinate as low as 4.5 °C, however optimal germination occurs between 20.2–29.3 °C (Soltani *et al.* 2006). Plants are self-fertilizing and flower about 50 days after emergence; however this is dependent on photoperiod and temperature (Singh 1997; Maiti and Wesche-Ebeling 2001). Flowers are perfect and vary in color from white, pink, purple or blue and mature pods carry one or two seeds (Moreno and Cubero 1978; Pundir *et al.* 1985). Some varieties possess glandular pubescence which secrets malic acid that can repel some insects (Maiti and Wesche-Ebeling 2001). The Desi and Kabuli type have fern-type leaf structure, but some Kabuli varieties have unifoliate leaf structure (Pundir *et al.* 1990). The adult plant has a bushy, semi-erect to semi-spreading growth type, and grows between 20 – 100 cm tall (Pundir *et al.* 1985; Singh 1997). Because chickpea has an indeterminate growth habit, maturity may be uneven. Chickpea is produced mainly for human food consumption (Wood and Grusask 2007). Both Desi and Kabuli type chickpeas are high in protein and fiber are also a source of calcium, iron, potassium, thiamin, riboflavin, niacin, Vitamin B6 and folate (Wood and Grusask 2007).

1.1.2. *Cicer* Classification and Origin

Chickpea originated from present day south-eastern Turkey and Syria (Van der Maesen 1987). Vavilov centers of origin include southwest Asia, the Mediterranean and Ethiopia (Van der Maesen 1987). Chickpeas belong to the family Fabaceae, tribe Cicereae and the genus Cicer (Kupicha 1977). Some closely related genera include: *Ononis, Medicago, Trigonella, Melilotus, Trifolium, Pisum, Lathyrus, Vicia* and *Lens* (Wojciechowski *et al.* 2004; Lavin *et al.* 2005). *Medicago* is a genera closely related to chickpea, and generally regarded as a model species in legumes (Lavin *et al.* 2005; Varshney *et al.* 2013). Cicer consists of 9 annual, 33 perennial and one unclassified species (Sethy *et al.* 2006). The *Cicer arietinum* primary gene pool consists of *Cicer reticulatum* and *Cicer echinospermum* (Ladizinsky and Adler 1976; Buhariwalla *et al.* 2005). Microsatellite (Sethy *et al.* 2006), inter-species simple sequence repeat (ISSRs) (Sudupak 2004; Choudhary *et al.* 2013) and amplified fragment length polymorphism (AFLP) (Nguyen *et al.* 2004) marker analyses suggested that *Cicer reticulatum* is the wild ancestor of today's cultivated chickpea *Cicer arietinum*.

1.1.3. Agricultural Production

The top ten producing countries of chickpea in 2011 in descending order are: India, Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, United States, Canada, and Mexico (FAOSTAT 2013). India is the world's leading chickpea producer and in 2011 produced 71% of the world's chickpea (Table 1.1) (FAOSTAT 2013). Even though Canada is a top 10 chickpea producing country, it still only produced 0.8% of the world's chickpea supply (Table 1.1) (FAOSTAT 2013). Canadian chickpea is grown predominantly in southeastern Alberta and southwestern Saskatchewan (Agriculture and Agri-food Canada 2008). Canadian chickpea production statistics are summarized in Table 1.2.

Table 1.1. 2011 World chickpea production by country (FAOSTAT 2013).

Country	Production (tonnes)	% World production
World Total	11,623,787.43	n/a
India	8220000.00	70.7
Australia	513338.00	4.4
Pakistan	496000.00	4.3
Turkey	487477.00	4.2
Myanmar	466738.00	4.0
Ethiopia	322839.00	2.8
Iran (Islamic Republic of)	290243.00	2.5
United States of America	97205.00	0.8
Canada	90800.00	0.8
Mexico	72143.00	0.6

Table 1.2. Canadian chickpea production statistics (Statistics Canada, 2012).

Crop Year	Production ('000 metric tonnes)	Exports ('000 metric tonnes)	Imports ('000 metric tonnes)
2009/2010	75.5	65.6	5.9
2010/2011	128.3	85.8	9.3
2011/2012	90.8	35.8	8.4
2012/2013	95.7	/	/

Chickpea agricultural production is as follows. The Brown/Dark Brown soil zone in Saskatchewan is ideal for chickpea (Padbury *et al.* 2002; Cutforth *et al.* 2007). The long tap-root system of chickpea makes the crop well suited for dry areas (Kashiwagi *et al.* 2006). Production usually includes a pre-seeding herbicide burn-off (e.g. Glyphosate) (Baker *et al.* 1996; Yadav 2007), seed treatment for *Pythium* (Leisso *et al.* 2009), fungicide treatment for *Ascochyta* blight (Banniza *et al.* 2011), and potentially desiccation to speed maturity . Main production constraints in western Canada are *Ascochyta* blight, late maturity and weed pressure (Agriculture and Agri-food Canada 2008; Siddique *et al.* 2011).

1.1.4. Weed Management

Chickpea is a poor weed competitor and there is potential for significant yield loss due to weed competition (Kantar *et al.* 1999; Felton *et al.* 2004). Weeds such as wild buckwheat (*Polygonum convolvulus*), *Kochia scoparia*, Russian thistle (*Salsola kali*), green foxtail (*Setaria viridis*), wild oats (*Avena fatua*), stinkweed (*Thlaspi arvense*), yellow foxtail (*Setaria glauca*), lady's thumb (*Polygonum persicaria*), Canada thistle (*Cirsium arvense*), sow thistle (*Sonchus arvensis*), dandelion (*Taraxacum* spp.), quackgrass (*Agropyron repens*) and volunteer crops are a problem in Western Canadian chickpea production (Agriculture and Agri-food Canada 2008). Reducing the spread of weeds, increasing crop competition or slowing weed adaptation to herbicides are agronomic techniques that together can manage weed pressure (Swanton and Weise 1991; Pande *et al.* 2007). Herbicides are one way to manage weeds, however post-emergence herbicide options for chickpea in Saskatchewan are limited to metribuzin (Sencor®, Bayer CropScience Canada) (Saskatchewan Ministry of Agriculture 2013; Taran *et al.* 2013). Additionally, metribuzin may cause leaf burn or stand thinning (Taran *et al.* 2013). Developing suitable varieties with different herbicide options would be desirable for Saskatchewan chickpea production. One option is to develop Group 2 resistant chickpea for which imidazolinone resistant germplasm has been identified (Taran *et al.* 2010) .

1.2. Imidazolinone Herbicides

1.2.1. Background

Group 2 herbicides include: imidazolinones (IMI), pyrimidinylthiobenzoates (PTB), sulfonylaminocarbonyltriazolinone (SCT), triazolopyrimidines (TP), and sulfonylureas (SU) (Cobb and Read 2010). Imidazolinones include: imidazolinones; imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin where imazethapyr, imazamox and imazamethabenz are registered herbicides in Saskatchewan (Weed Science Society of America 2007; Cobb and Read 2010; Saskatchewan Ministry of Agriculture 2013). All imidazolinones possess an imidazole ring and depending on class, will possess a unique R group or secondary cyclic ring (Tan *et al.* 2005; Weed Science Society of America 2007). Agronomic information for IMI herbicides from the Saskatchewan Guide to Crop Protection are summarized in Table 1.3. Imidazolinone herbicides are not currently registered for use on chickpea in Canada, but are registered for used on field pea, dry bean and soybean pulse crops.

Table 1.3: Summary of weed control characteristics and agronomic use of different imidazolinone herbicides registered for use on Saskatchewan field crops (Saskatchewan Ministry of Agriculture 2013)

IMI herbicide	Registered Crop Use	Formulation	Weeds Controlled or Suppressed	
Imazethapyr: BASF Pursuit® Univar Gladiator® Viterra Multistar®	Barley Spring Wheat Sunflower	300g L^{-1} imazamethabenz	Stinkweed Wild mustard	Buckwheat Volunteer Canola (not CLEARFIELD)
Imazamox	CLEARFIELD crops: Sunflower Lentil Canola Oilseed mustard	70% imazamox	Barnyard grass Cleavers Cow cockle Green foxtail Green smartweed Japanese brome Kochia Lamb's-quarters Persian darnel Redroot pigweed Shepherd's-purse Stinkweed	Volunteer barley Volunteer canaryseed Volunteer canola (not CLEARFIELD) Volunteer oat Volunteer wheat (not CLEARFIELD) Wild buckwheat Wild mustard Wild oat Yellow foxtail
Imazamox and Imazethapyr	CLEARFIELD crops: Sunflower Lentil Canola Oilseed mustard	35% imazamox and 35% imazethapyr	Barnyard grass Green foxtail Persian darnel Volunteer cereals (not CLEARFIELD) Wild oat Chickweed Cleavers Flixweed Green smartweed	Hemp-nettle Kochia Lamb's quarters Redroot pigweed Russian thistle Shepherd's purse Stinkweed Stork's bill Wild Buckwheat Wild mustard
Imazethapyr	Field pea Dry bean Soybean Alfalfa Chickling vetch	240g L^{-1} imazethapyr	Chickweed Cleavers Green foxtail Hemp-nettle Redroot pigweed Shepherd's-purse Smartweed	Stinkweed Volunteer canola (not CLEARFIELD) Wild buckwheat Wild mustard Wild oats Common groundsel

1.2.2. AHAS/ALS Enzyme

All Group 2 herbicides inhibit *acetohydroxyacid synthase* (AHAS) also known as *acetolactate synthase* (ALS) (previously classified at E.C. 4.1.3.18 now E.C. 2.2.1.6) (Shaner *et al.* 1984; Weed Science Society of America 2007; Moss 2013). The reaction it catalyzes is: 2 pyruvate = 2-acetolactate + CO_2 and is involved in branch chain amino acid biosynthesis (Figure 1.1) (Duggleby and Pang 2000; Zhou *et al.* 2007; Moss 2013). The systematic name *pyruvate:pyruvate acetaldehydetransferase* (decarboxylating) and other names include: *α-acetohydroxy acid synthetase; α-acetohydroxyacid synthase; α-acetolactate synthase; α-acetolactate synthetase; acetohydroxyacid synthetase; acetohydroxyacid synthase; acetolactate pyruvate-lyase* (carboxylating); and *acetolactic synthetase* (Moss 2013). The AHAS enzyme is found in archaea, bacteria, fungi, algae and plants, however this review will focus on plant AHAS. The AHAS enzyme forms a tetramere with two identical regulatory and catalytic subunits (Lee and Duggleby 2001; Lee and Duggleby 2002; McCourt *et al.* 2006; Duggleby *et al.* 2008). Lee and Duggleby (2001) studied the small regulatory subunit of *Arabidopsis thaliana* and determined its role in branch chain amino acid (BCAA) feedback regulation AHAS. The regulatory subunit is about 180 amino acids long consisting of a chloroplast targeting peptide with two identical regions joined by a linker sequence (Lee and Duggleby 2001). Work with yeast and *Arabidopsis thaliana* AHAS gave insight to the AHAS catalytic structure; consisting of three domains with a C-terminal tail looping over the active site (Pang *et al.* 2002; McCourt *et al.* 2006).

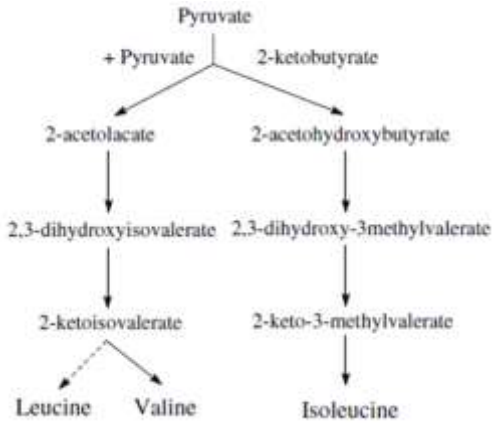

Figure 1.1 Biosynthesis of branched chain amino acids (Zhou *et al.* 2007)

1.2.3. Mode of Action

Canada uses a herbicide classification system based on mode of action (Mallory-Smith and Retzinger 2003). IMI herbicides are absorbed through leaves or roots depending on IMI formulation, and translocation occurs through either the xylem and phloem (Foley and Bauman 1995; Weed Science Society of America 2007). Group 2 herbicides inhibit AHAS by binding to residues in a channel leading to the AHAS active site, but does not directly compete for the active site (McCourt *et al.* 2006; Duggleby *et al.* 2008). Key herbicide binding sites have been identified: 16 residues contact with SUs and 12 residues contact with IMIs and 10 of these sites are shared between SUs and IMIs (McCourt *et al.* 2006). These amino acids are useful when developing new Group 2 resistant plant varieties. Even though the mechanism of herbicide binding and inhibition has been identified, cause of plant death is less understood. It is suggested that Group 2 herbicides cause plant death by impeding BCAA metabolism and biosynthesis, along with other secondary mechanisms (Manabe *et al.* 2007; Zhou *et al.* 2007).

1.2.4. Agronomic Advantages and Disadvantages

A study conducted in Turkey determined that the use of herbicides like imazethapyr on chickpea provide good weed management option (Kantar *et al.* 1999). Even though this study also showed that hand weeding provided similar weed control, the man power required to spray herbicide as compared to hand weeding is considerably less (Kantar *et al.* 1999). Some characteristics that make IMI herbicides desirable for resistance breeding include:

- low mammalian toxicity – oral LD50 >5000 mg kg^{-1} , dermal LD50 >2000 mg kg^{-1} (depending on imidazolinone formulation) (Weed Science Society of America 2007)
- non-corrosive, non-flammable (Weed Science Society of America 2007)
- non-contaminate of ground water (Weed Science Society of America 2007)
- targets broadleaf weeds (Saskatchewan Ministry of Agriculture 2013)
- effective at a low dose (17.3g/acre Odyssey®, 11.7g/acre Solo®) (Saskatchewan Ministry of Agriculture 2013)

A major disadvantage of Group 2 herbicides is numerous (131) resistant weed species (Heap 2013). Tranel and Wright (2002) extensively reviewed this issue and concluded that extensive use of Group 2 herbicides, simple mode of inheritance of resistance (Section 2.3.2), multiple possible genetic point mutations causing resistance (Section 2.3.3) and lack of fitness cost are the reasons for the high number of resistant weed species. Additionally, a single point mutation may result in cross resistance , especially when AHAS Trp574 is substituted (Tranel and Wright 2002). Another disadvantage to some IMI formulations is the possibility for soil residual activity that may reduce productivity following this season's crop. Some environmental conditions that slow herbicide degradation include: drought, prolonged cool weather, acidic (<pH 6.5) soil, high rates or multiple treatments with Group 2 herbicides (Geisel *et al.* 2008).

1.2.5. Cross Resistance of AHAS Inhibiting Herbicides

Herbicide cross resistance has both beneficial and unfavourable aspects. On one side, crops resistant to multiple herbicides provide added flexibility to agronomic management for producers. In cultivated sunflower, various Group 2 herbicide resistant varieties have been genetically characterized (Sala *et al.* 2012a). A recent study has identified and genetically characterized a point mutation responsible for IMI, SU, TZ and POB cross resistance in sunflower (Sala and Bulos 2012). Through molecular breeding techniques like marker assisted selection (MAS), Group 2 resistance could be efficiently integrated into new sunflower varieties (Kolkman *et al.* 2004; Sala *et al.* 2012a). However, when weed species develop cross resistance, chemical weed management may become difficult. For example, wild radish (*Raphanus raphanistrum* L.), a weed species in Australia, has been found to be resistant to SU, TP and IMI making management with Group 2 herbicides no longer an economical option. Group 1 and 2 weed herbicide resistance in Western Canadian provinces was studied and reviewed by Beckie and Tardif (2012). They determined that economically damaging weeds like *Kochia*, wild mustard, field pennycress, *Galium spp*., common chickweed, and common hemp nettle all had varying levels of Group 2 cross resistance.

1.3. Developing Imidazolinone Herbicide Resistance

1.3.1. Plant Breeding Techniques

Herbicide resistant varieties can be developed through classical breeding using resistant germplasm (e.g. gene banks) or through tissue culture procedures, chemical mutagenesis using ethyl methanesulfonate (EMS), sodium azide (NaN3) or irradiation to induce mutation. Tar'an *et al*. (2010) identified four chickpea lines (ICC2242, ICC2580, ICC3325 and ICCX860047–9)

resistant to IMI herbicides that could be used in classical chickpea breeding. Examples of Group 2 herbicide resistance species developed through the use EMS mutagenesis include: *Arabidopsis thaliana* and *Medicago truncatula* (Haughn and Somerville 1986; Heap 2000) and wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) using sodium azide (Newhouse *et al.* 1992; Li *et al.* 2008; Lee *et al.* 2011). Toker *et al.* (2012) used 300 and 400 Gy gamma ray irradiation to induce mutation causing IMI resistance in *Cicer reticulatum* L. Tissue culture and somatic cell IMI resistance selection was utilized by Wright and Penner (1998) to develop IMI resistant sugarbeet (*Beta vulgaris)*. Once resistant lines have been developed, conventional breeding methods can be implemented to incorporate the trait from a mutant line into an agronomically adapted variety (Salimath *et al.* 2007).

1.3.2. Inheritance of Resistance

Through conducting herbicide studies on segregating populations (usually F_2), gene action and mode of inheritance of Group 2 herbicide resistance can be determined. In *Arabidopsis thaliana*, sugarbeet (*Beta vulgaris*), *Medicago truncatula*, and barley (*Hordeum vulgare* L.) Group 2 herbicide resistance is controlled by a single semi-dominant to dominant gene (Haughn and Somerville 1990; Wright and Penner 1998; Oldach *et al.* 2008; Lee *et al.* 2011). Sunflower (*Helianthus annuus* L) showed a unique mode of inheritance, where the *AHAS1–1* allele is co-dominant to recessive depending on IMI dose and *AHAS1–3* is semi to fully dominant but dominant over *AHAS1–1* (Sala *et al.* 2012b).

1.3.3. Previous Genetic Characterization (*AHAS* sequencing)

Lee and Duggleby (2001; 2002) studied the regulatory subunit's structure. The regulatory subunit consists of 491 amino acids, consisting of chloroplast targeting peptides (~80 aa), two duplicate regions (~180 aa) separated by a linker (~50 aa). However, most research characterizes

the AHAS catalytic subunit as mutations affecting Group 2 herbicide binding sites may result in a resistant phenotype (McCourt *et al.* 2006). Work with *Arabidopsis thaliana* mutants characterized AHAS genes and mechanism for Group 2 resistance (Sathasivan *et al.* 1990; Sathasivan *et al.* 1991). and The *Arabidopsis thaliana AHAS* sequence (NCBI reference NM_114714.2) is 2270 bp long, and contains a mutation causing IMI resistance (Sathasivan *et al.* 1991). A point mutation in *AHAS* may alter sensitivity to Group 2 herbicides and many point mutations causing IMI resistance have been identified (Ott *et al.* 1996; Duggleby *et al.* 2003; Tan *et al.* 2005; Beckie and Tardif 2012). Most amino acid substitutions occur at Ala122, Pro197, Ala205, Trp574, and Ser653 (alignment based on the *Arabidopsis thaliana* amino acid sequence NCBI reference NM_114714.2) (Tan *et al.* 2005). The International Survey of Herbicide Resistant Weeds provides an extensive database is available outlining amino acid mutation, species, AHAS inhibitor class and corresponding research (Heap 2013). Generally, the *AHAS* sequence is about 2200bp/670aa long (*Arabidopsis thaliana* NCBI reference NM_114714.2) with no introns (Kolkman *et al.* 2004).

Both Ala122 and Ala205 point mutations result in IMI resistant phenotypes (Heap 2013). Work by Li *et al.* (2008) characterized an Ala 122 mutation on chromosome 6D conferring IMI resistance in wheat (*Triticum aestivum* L.). A study looking at wild radish in Australia (*Raphanus raphanistrum*) showed that an Ala122 mutation in *AHAS* resulted in SU, TP and IMI cross resistance (Han *et al.* 2012). Sunflower (*Helianthus spp*), Redroot Pigweed (*Amaranthus retroflexus*) and Eastern Black Nightshade (*Solanum ptychanthum*) have an Ala205→Val point mutation consistent with IMI resistance (White *et al.* 2003; McNaughton *et al.* 2005; Ashigh and Tardif 2007). Sunflower showed both IMI resistance and partial SU resistance (White *et al.* 2003).

Work with *Arabidopsis thaliana* mutants showed *AHAS* mutations causing Group 2 resistance. SU resistant *Arabidopsis thaliana* mutants (Haughn and Somerville 1986) were the results of a C→T point mutation causing a Pro197→Ser substitution (Haughn *et al.* 1988; Mourad and King 1992).

A Trp574 substitution has been found to cause Group 2 herbicide cross resistance in various weed species (Heap 2013). Because cross resistance may result from this single substitution, it makes it a desirable target when developing herbicide resistant lines (Bernasconi *et al.* 1995). In wild mustard (*Sinapis arvensis*), Trp574→Leu mutation confers resistance to four classes of Group 2 herbicides (Christoffers *et al.* 2006).

The Ser653 amino acid substitution has been identified and characterized in *Arabidopsis thaliana,* barley (*Hordeum vulgare* L) and wheat (*Triticum aestivum* L.). Through research with the imazapyr resistant *Arabidopsis thaliana* GH90 mutant, a G1958→A nucleotide mutation at the *csr1–1* locus results in Ser653→Asn substitution conferring IMI resistance (Haughn and Somerville 1990; Sathasivan *et al.* 1991; Mourad and King 1992). Additional transcription profiling determined that the *csr1–1* locus is the only target of IMI herbicides in *Arabidopsis thaliana* (Manabe *et al.* 2007). Other Ser653 mutations in *Arabidopsis thaliana* were characterized and resulted in varying levels of IMI and SU resistance (Lee *et al.* 1999). In barley (*Hordeum vulgare* L) imidazolinone resistance was caused by an *AHAS* point mutation G→A at position1742 (Lee *et al.* 2011). A Ser→Asn substitution was also characterized in wheat (Pozniak *et al.* 2004).

2. Materials and Methods

Preparation of plant material was conducted similarly across all research components. Desi type seeds were scarified using tweezers about 24 hr before seeding. Sunshine mix #4 (Sun Grow, Seba Beach, AB) was used, but washed using warm water 4 – 5 times before seeding and allowed to drain a minimum of 2hr. Seeds were also treated using a mixture of Fludioxonil, Metalaxyl-M S-isomer and Thiabendazole (Apron FL®, Syngenta Canada INC) to prevent root rot. Plants growing in the College of Agriculture and Bioresources Greenhouses were maintained at an average of 21.09 °C air temperature, 21.41 integrated photosynthetic radiation, and 44.0% relative humidity in winter (January 2012, Average Greenhouse statistics, Zone E) and 26.21 °C air temperature, 52.08 integrated photosynthetic radiation, and 80.42% relative humidity in summer months (July 2012, Average Greenhouse statistics, Zone E). All greenhouse environmental condition statistics may be found at http://agbio.usask.ca/research/centres-facilities/greenhouses.php. Two growth chambers at the Controlled Environment Facility (Phytotron) in the College of Agriculture and Bioresources were used in herbicide screening experiments (Section 2.3.2) and RIL generation advancement. Chamber 1 – 33 was maintained at 22°C/14hr day and a 16°C/10hr night while chamber 1 – 4 was maintained at 24°C/14hr day and a 16°C/10hr night.

2.1. Component 1: Sequencing *acetohydroxyacid synthase* (*AHAS*) gene in chickpea

2.1.1. Plant Material

Seven chickpea varieties were used for sequencing and comparison of the *AHAS* gene; four are IMI susceptible and three are IMI resistant (Table 2.1). Plants used for DNA samples were grown in in the College of Agriculture and Bioresources Greenhouses or Phytotron growth chambers.

Table 2.1. Characteristics of chickpea varieties used for *AHAS* genetic characterization

Variety	IMI Response	Market Class
Myles	Susceptible	Desi
CDC Corinne	Susceptible	Desi
CDC Frontier	Susceptible	Kabuli
CDC Luna	Susceptible	Kabuli
ICCX860047-9	Resistant	Desi
CDC Cory	Resistant	Desi
CDC Alma	Resistant	Kabuli

2.1.2. PCR and Sequencing Preparation

About 1 – 2 of the youngest leaves were harvested before flowering for DNA isolation. Tissue was placed in a 2ml micro-centrifuge tube with four glass beads, then flash-frozen using liquid nitrogen and stored at -80°C. Tissue was freeze-dried for 24 – 48hr then ground with a mixer mill. The DNeasy® Plant Mini Kit (QIAGEN Inc. Mississauga, ON), was used for DNA isolation

Sequencing of *AHAS2* used primers designed from the *Cicer arietinum* transcriptome database (ICC4958) (Garg *et al.* 2011). The Primer3 online program (WWW. primer tool; Whitehead Institute for Biomedical Research, 1998) was used to design primers to amplify *AHAS2* in three segments, each of about 700 – 800 bp in length. Primers were chosen based on clarity of the bands in agarose gels. The following is sequence information for each primer pair:

Primer Name	Nucleotide Sequence
Ca – AHAS2 L3	ACAATAGAGATTTTAAAGGCCTGCAT
Ca – AHAS2 R1115	CAAAAGATCDCTCTTATCAACAGCAT
Ca – AHAS2 L33	GAACTGTTTCATTCAACAGAAAAATG
Ca – AHAS2 R1808	AGCATCTGCAAATTTCAACATATTAG

Ca – AHAS2 L1069	TGGAACTGTTTATGCTAATTATGCTG
Ca – AHAS2 2239R	GATTTTCAGTAACCAAATAACCAAGG

Using the chickpea *AHAS2* consensus sequence as query, the homologous gene *AHAS1* (amino acid translation 80% similar) was found in the CDC Frontier draft genome sequence (Varshney *et al.* 2013). CDC Frontier *AHAS1* sequence was then used to design primers to sequence *AHAS1* in IMI resistant and IMI susceptible chickpea genotypes. The following is sequence information for each primer pair to amplify *AHAS1* gene:

Primer Name	Nucleotide Sequence
Ca – AHAS1-L33	CGCATTACCATCDCACDCAC
Ca – AHAS1-R1053	CTAGGTAGTTACCCTGTTGGAGGAG
Ca – AHAS1-L54	GTAAACTCGAACTCCATCATTCATT
Ca – AHAS1-R1053	CTAGGTAGTTACCCTGTTGGAGGAG
Ca – AHAS1-L696	AGATCCATCDCAAAGCATAACTACC
Ca – AHAS1-R1495	CTAACAATAGCATCDCCATTTGTCA
Ca – AHAS1-L841	ATTGATTCGGCTGAAATTGG
Ca – AHAS1-R2292	CGATCDCTTCAACCTGAATCTC
Ca – AHAS1-L1173	GATGATCGTGTAACTGGGAAATTAG
Ca – AHAS1-R2289	TCDCTTCAACCTGAATCTCDCTACA
Ca – AHAS1-R1495	CTAACAATAGCATCDCCATTTGTCA
Ca – AHAS1-R2289	TCDCTTCAACCTGAATCTCDCTACA

These primers were used in Polymerase Chain Reaction (PCR) to amplify the *AHAS1* and *AHAS2* fragments from IMI susceptible: Myles, CDC Corinne, CDC Luna, CDC Frontier and IMI resistant ICCX860047–9, CDC Cory and CDC Alma. PCR reaction conditions were optimized to increase amplification. The PCR components for a 25 µl single reaction were:

4.0 µl of 10 ng µl-1 genomic DNA template

1.0 µl of 10 µM primer

2.5 µl Taq buffer

2.5 µl of 15mM MgCl

0.5 µl of 10mM dNTP

0.2 µl (1 unit) Genscript Taq polymerase

13.3 µl of distilled autoclaved water

The samples were amplified using a BIO-RAD-C1000TM or PTC-100® thermocycler with the following programs:

Step 1 – 2 min at 95°C initial denature

Step 2 – 30 sec at 94°C denature

Step 3 – 1 min at 60°C annealing

Step 4 – 1.5 min at 72°C extension

Step 5 – return to step 2 for 34 additional cycles

Step 6 – 10 min at 72°C final extension

Step 7 – 8°C until samples removed from thermocycler

PCR products were dyed using GenScript GelRed™ loading dye then separated and inspected on a 1.5% agarose gel electrophoresis with Tris-acetate (TAE) buffer. The QIAGEN® QIAquick®

Gel extraction kit (QIAGEN Inc. Mississauga, ON) was used to extract and purify DNA of the correct size (about 1000bp). Eluded DNA was sent for Sanger sequencing at the National Research Council Canada on the University of Saskatchewan Campus (Saskatoon, Saskatchewan). To locate the SNPs and compare sequence data, Sequencher® 5.0 software (Gene Codes Corporation, Ann Arbor, MI United States) was used.

2.1.3. KASP SNP genotyping preparation

Plant samples for DNA sampling were prepared the same as for the *AHAS* sequencing plant samples (Section 2.1.1). Two of the youngest leaves were harvested for DNA using a modified CTAB procedure (Doyle and Doyle 1987). DNA was quantified using a FLUOstar Omega Fluorometer (BMG LABTECH Ortenberg,Germany) and diluted to 10 ng/µl.

The KBioscience Allele-Specific PCR Genotyping system (KBioscience Ltd., Hoddesdon, UK) was used to develop and test SNP markers for selecting IMI resistance genotypes in the RIL population (Figure 2.1). Using chickpea *AHAS1* sequence data (Section 2.1.2) primers targeting the point mutation in *AHAS* responsible for IMI resistance were designed using Primer Picker Software offered by KBioscience (http://www.kbioscience.co.uk/primer-picker/).

The reaction was run on a StepOnePlus™ Real-Time PCR system with the following program:

Step 1 – 60 °C for 30 sec (florescence read)

Step 2 – 95 °C for 10 min

Step 3 – 95 °C for 15 sec

Step 4 – 60 °C for 1 min (repeat step 3 – 4, 40x) (florescence read)

Step 5 – 60 °C for 30 sec (florescence read)

Fluorescence data and SNP calls were made using StepOneTM Software v2.1 (Applied Biosystems. Foster City, CA, USA). The first and final florescence reads were used in SNP

calling. SNP calls were made based on parental florescence reads and data displayed on the allelic discrimination plot generated by the software. This data was compared to herbicide rating data (Section 2.3.2)

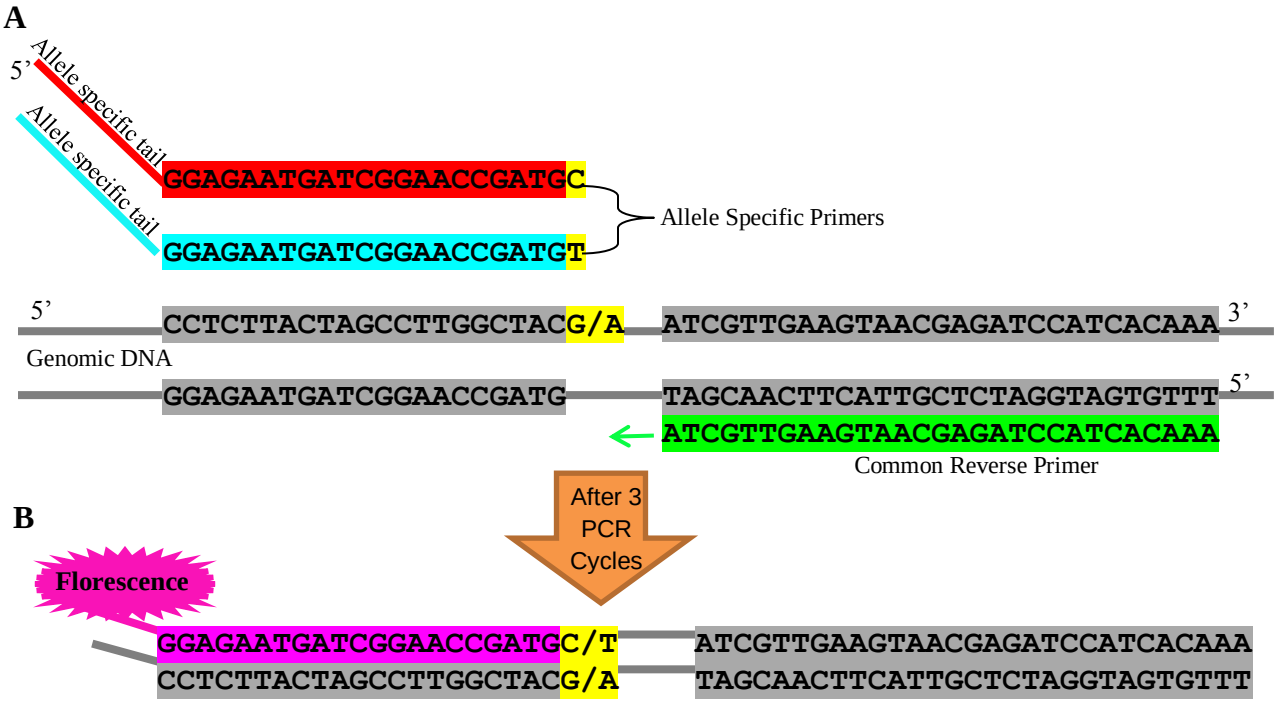

Figure 2.1 Diagrammatic representation of A) KASP reaction components and B) final signal generation step of KASP SNP genotyping. Genomic DNA is used in allele specific PCR resulting in bi-allelic SNP calling through FAM™ and HEX™ fluorescently labeled oligonucleotides. The point mutation site that confers to IMI resistance in chickpea is indicated in yellow

	Allele specific tail	Compliment to Genomic DNA (Mutation Site)
Allele Specific Primer 1	GAAGGTGACCAAGTTCATGCT	GGAGAATGATCGGAACCGATGC
Allele Specific Primer2	GAAGGTCGGAGTCAACGGATT	CGGAGAATGATCGGAACCGATGT
Common/Reverse Primer	TTTGTGATGGATCTCGTTACTTCAACGAT	

2.2. Component 2: Synteny of *AHAS* Gene

2.2.1. Sequencing Lentil *AHAS* Gene

Lentil DNA from CDC Redberry (IMI susceptible) and CDC Impact (IMI resistant) were used to sequence the gene homologous to *AHAS1* in chickpea. A modified CTAB procedure was used to extract the DNA (Doyle and Doyle 1987).

Using the Primer3 online program (WWW. primer tool; Whitehead Institute for Biomedical Research, 1998) three primer pairs were designed from Lc0.3_scaffold187143 scaffold of lentil cDNA sequence (http://knowpulse2.usask.ca/portal/). This scaffold was found to be homologous with the chickpea *AHAS1* when compared using NCBI BLAST® (query cover 88%, E value = 0.0). Primer sequence information is as follows:

Lc – AHAS1 L33	AACCAGCTATGAGTTGACDCGA
Lc – AHAS1 R1297	CAACAAACCGGTTCAATTCC
Lc – AHAS1 L873	CGATGCTTTGATGGACAGTG
Lc – AHAS1 L1669	CATTCAACTCCTGCCTCCAT
Lc – AHAS1 L1434	ACTTGCTTTCGGGGTTAGGT
Lc – AHAS1 R2721	CTCAATCDCTTCAGCCTCAATC

Direct sequencing and sequence analysis was conducted using the same protocol as in chickpea (Section 2.1.2).

2.2.2. Cluster Analysis

Chickpea and lentil *AHAS* sequences were combined with other publicly available sequences to study the synteny of the *AHAS* gene. Consensus chickpea *AHAS* sequences were used as query to search NCBI BLAST® online database and homologous sequences were retrieved. The *AHAS1*

and *AHAS2* from the CDC Frontier genome sequence, the *AHAS2* sequence from *Cicer reticulatum* and the *AHAS2* sequence of ICC4958 (*Cicer arietinum*) from the chickpea transcriptome database (http://59.163.192.90:8080/ctdb/) and the lentil *AHAS1* sequences were used in conjunction with the following homologous *AHAS* sequences:

Legume Species:

Cicer arietinum (ICC4958) (Garg *et al.* 2011), 2294 bp

Cicer reticulatum (CrTC30570) (Garg *et al.* 2011). 2177 bp

Glycine max (FJ581423.1), 1938 bp

Glycine max AHAS 2 (XM_003545859.1), 2146 bp

Lotus japonicus (AK339751.1), 2197 bp

Medicago truncatula (XM_003593479.1), 2258 bp

Medicago truncatula 'Caliph' (EU292216.1), 2052 bp

Medicago littoralis 'Angel' (EU292213.1), 2165 bp

Phaseolus vulgaris 'Olathe Pinto' (GQ466185.1), 1947 bp

Other Plant Species:

Helianthus annuus AHAS1 (AY541451.1), 1968 bp

Helianthus annuus AHAS2 (AY541457.1), 1947 bp

Sinapis arvensis AHAS1-R (AY954041.1), 380 bp

Sinapis arvensis AHAS1-R (AY954042.1), 380 bp

Molecular Evolutionary Genetics Analysis (MEGA) (Tamura *et al.* 2011) software was used for sequence multiple alignment, cluster analysis and phylogenetic tree construction. ClustalW in

MEGA was used to conduct the initial multi-sequence alignment. A phylogenetic tree was computed using the Neighbor–joining algorithm with Bootstrap confidence levels (500 replications).

2.3. Component 3: Inheritance and Molecular Mapping of *AHAS1* Gene

2.3.1. Plant Material

A population of recombinant inbred lines (70 RILs) from a cross of ICCX860047-9 (IMI resistant) and CDC 512- 51 (IMI susceptible) advanced through single seed descent to F7 and F8 (~98% homozygous) was used to examine phenotypic response to IMI herbicide.

2.3.2. Screening RILs for Herbicide Resistance

Ten seeds per RIL were scarified and pre-germinated in a petri dish with dampened filter paper for 24 – 48hr. When plants were between the 2 – 6 leaf stage (10 – 14 days after seeding), a spray cabinet (Taran *et al.* 2010) was used for herbicide application with the following settings: Even-Spray nozzle 8001 EVS, operated at 240kPa, spray calibrated to 100 L ha^{-1}. Plants were sprayed with Solo® (70% imazamox) at a rate of 20.22g a.i. ha^{-1} (11.7 g. Solo®/acre) with water volume of 40L/acre. In the spray cabinet, a 200ml solution was used to spray the plants, meaning that 0.116g Solo®/200mL water was used. Also adjuvant Merge® was used at a rate of 0.5 mL/100mL solution. Herbicide rating of either resistant or susceptible was taken at 7, 14 and 28 days after herbicide treatment (DAT). CDC Corinne (IMI susceptible) and CDC Cory (IMI resistant) were the check varieties used in herbicide ratings (Figure 2.2). A resistant rating was given if there were no changes in morphology. A susceptible plant showed typical herbicide injury symptoms such as stunted growth, chlorosis, necrosis and small needle-like leaf development. Chickpea exhibits unique herbicide injury symptoms that varied between necrosis,

increased branching, yellowing of plant tissue, needle-like leaf development and stunted growth (Taran *et al*. 2010)**.** Phenotypic data from this study was used in a chi-square analysis to determine the mode of inheritance.

Figure 2.2 Chickpea check varieties in response to IMI herbicide at 7 days after treatment with Solo® (imazamox) at a rate of 20.22 g a.i. ha^{-1} (11.7 g. Solo®/acre), treatment was applied at plant age between 17 – 21 days after seeding A) left, CDC Cory IMI resistant B) right, CDC Corinne IMI susceptible.

2.3.3. SNP Illumina GoldenGate® Assay

The same RIL population was used for SNP genotyping and molecular mapping. A modified CTAB procedure was used for DNA extraction of each RIL (Doyle and Doyle 1987). DNA was quantified using a FLUOstar Omega Fluorometer (BMG LABTECH Ortenberg, Germany) and diluted to 50 ng/µl. Then 20 µl of 50 ng/µl DNA from each RIL and both parental lines CDC 512 -51 and ICCX860047-9 were transferred to a 96 well plate. The National Research Council

(Saskatoon, Saskatchewan) carried out the 1536 Illumina GoldenGate® Genotyping Assay previously designed for chickpea.

The Illumina GoldenGate® SNP genotyping platform is a high-throughput SNP genotyping system which can be used to map genes of interest (Fan *et al.* 2006). The first step involves activating high quality DNA (50ng ul^{-1}) to streptavidin or biotin coated magnetic beads. Allele specific oligonucleotides were designed specific to each SNP location, where each oligonucleotide binds depending on the nucleotide at the target site. The Locus-Specific oligonucleotide will bind downstream of the target site. This provides a template for universal PCR where fluorescently labeled primers (Cy3 and Cy5) are used. Products hybridize to a Beadchip and an Illumina HiScanSC BeadArray Reader records fluorescence values to be analyzed (Illumina, San Diego, CA).

2.3.4. Linkage Analysis

Raw marker data were received from the National Research Council (Saskatoon, Saskatchewan) who carried out the Illumina GoldenGate® Genotyping Assay (Illumina San Diego, CA USA). The Illumina GenomeStudio ver 2010.1 Data Analysis Software (Illumina San Diego, CA USA) was used to analyze the SNP genotypes. A SNP graph at each SNP locus was generated by the software and individually inspected and classified as monomorphic, polymorphic, heterozygous, dominant or failed. Only polymorphic markers between the two parents were used for molecular mapping.

The data was then exported into Microsoft Excel for initial sorting. Next, SNP markers were sorted into linkage groups using CarthaGène with the minimum logarithm of odds (LOD) threshold of 6.0 with a distance threshold of 0.3 recombination fraction (Institut National de la

Recherche Agronomique) (Institut National de la Recherche Agronomique; de Givry *et al.* 2005). The Kosambi mapping function was used to create linkage maps (Kosambi 1943) which were aligned with the consensus map developed using ICCV96029xCDC Frontier population (Tar'an *et al.* 2007; Anbessa *et al.* 2009) using MapChart version 2.2. (file:///C:/Program%20Files%20%28x86%29/MapChart/MCManual.htm#_Toc120251480).

2.4. Component 4: AHAS Enzyme Activity

A colorimetric assay was used to compare AHAS enzyme activity in response to IMI treatment in CDC Frontier (IMI susceptible) and CDC Cory (IMI resistant). Both *in vivo* and *in vitro* assays used the following procedure: Tissue samples were collected and flash frozen using liquid nitrogen in a sterile DNAse/RNase free 1.7 ml microcentrifuge tube. Leaf tissue was prepared by grinding it to powder in liquid nitrogen and transferred to sterile DNAse/RNase free 2 ml microcentrifuge tubes then stored at -20 °C. Next, 1.5mL protease inhibitor cocktail (Sigma-Aldrich Co. LLC) containing methanol and protease inhibitor was added to tissue, mixed by vortex and incubated at -20 °C for 5min. Samples were centrifuged at 16,000g for 5 min at 4 °C and supernatant was discarded. The protease and centrifuge steps were repeated 4 – 5 times. Next, 1.5 mL acetone pre-chilled at -20 °C was added, mixed by vortex , and incubated at -20 °C for 5min. Samples were centrifuged at 4^{o}C for 5 min at 16, 000 *x g* then supernatant discarded and pellet dried for 20 min.

To examine AHAS activity over time, a colorimetric assay was modified from Singh and Shaner (1988). This assay involves the conversion of the AHAS product acetolactate to acetoin then incubation with napthol and creatine resulting in a pink coloring. The *in vivo* assay used CDC Frontier and CDC Cory treated with Solo® (70% imazamox) at a rate of 20.22g a.i. ha^{-1} (11.7 g.

Solo®/acre) in a spray cabinet with tissue collected at 0, 2, 4, 6, 12, 24, and 120 hr (5 days) after herbicide treatment. Three biological replicates were used per genotype per time increment and each biological replicate consisted of two technical replicates. Total protein was extracted as described at the beginning of this section. Total protein from CDC Frontier and CDC Cory measuring 3.1 mg of was combined with 200µl incubation buffer and was maintained at 37^{o}C for 1 hour. The incubation buffer consisted of 50 mM Potassium phosphate buffer (pH 7.0), 100mM Sodium pyruvate, 10mM Magnesium chloride, 1mM Thiamine pyrophosphate and 1uM FAD. To stop the reaction 0.85% H_2SO_4 was used, then decarboylation at 65^{o}C for 15 min was allowed. The product was incubated with creatine (0.17% in 2N NaOH) and (1.7% in 2N NaOH) alpha-napthol at 60^{o}C for 15 min. Then 200µl of the solution was transferred to a 96 well microliter plate and absorbance at 520nm was measured.

The *in vitro* assay used tissue collected from untreated CDC Frontier and CDC Cory plants and was prepared as in the *in vivo* study. However, concentrations of 0, 3, 6, 8, 10, 12, 14 µM imazamox PESTANAL®, analytical standard (Sigma-Aldrich Co. LLC) was added to the incubation buffer. Two biological replicates were used per genotype per IMI treatment and each biological replicate consisted of two technical replicates. Absorbance at 520nm was recorded using a FLUOstar Omega Fluorometer (BMG LABTECH Ortenberg, Germany). Individual controls were prepared for each experiment, and consisted of all reagents without protein. Each absorbance value was an average of the two technical replicates. Data was analyzed in Microsoft Excel.

3. Results

3.1. Component 1: Sequencing *Acetohydroxyacid Synthase* (*AHAS*) Gene

3.1.1. Sequence Analysis

Both chickpea *AHAS1* and *AHAS2* were direct sequenced from PCR fragments amplified using primers designed from the chickpea genome or transcriptome sequence database (Garg *et al.* 2011; Varshney *et al.* 2013). Nucleotide sequences from the CDC Frontier genome *AHAS1* and *AHAS2* were aligned using the NCBI BLAST® multiple alignment tool. Identities of 1409/1739 were observed, meaning *AHAS1* and *AHAS2* are 81% identical (amino acid alignment = 80% identity) suggesting that *AHAS1* and *AHAS2* are homologous. When comparing sequences of *AHAS2* across IMI susceptible and resistant chickpea genotypes, there was no mutation consistent with the herbicide resistance. *AHAS2* sequence analysis was not included in this report; but *AHAS2* sequence is available in Appendix 1 and 2. The following is the sequencing results and analysis of *AHAS1*.

Seven chickpea genotypes were used in sequence analysis. The genotypes included both IMI susceptible (Myles, CDC Frontier, CDC Corinne and CDC Luna) and IMI resistant (ICCX860047-9, CDC Cory and CDC Alma) varieties. Both nucleotide and amino acid position numbers are based on *Arabidopsis thaliana* (NCBI reference NM_114714.2) *AHAS* sequence which is 2270 nucleotides or 670 amino acids long. *Medicago truncatula* 'Caliph' (EU292216.1) and *Medicago littoralis* 'Angel' (EU292213.1) were aligned as a legume species reference. The consensus chickpea IMI susceptible *AHAS1* sequence is 2186 bp long and 659 aa long and the consensus chickpea IMI resistant *AHAS1* sequence is 2147 bp long and 651 aa long, both with no introns (Appendix 4, 5). A point mutation at base pair 675 from C→T that resulted in the amino acid substitution Ala205→Val205 was found (Figure 3.1). Amino acid alignment shows that the

upstream 5' region of the IMI resistant consensus was not completely sequenced, missing 8 amino acids (Appendix 5). However areas containing common mutation site were sequenced (highlighted areas, Appendix 5). The Ala205 mutation is consistent with other known mutations causing Group 2 herbicide resistance (Tan *et al.* 2005; Beckie and Tardif 2012).

A

Response to IMI herbicide	Genotype	Nucleotide Sequence (675 bp)
Susceptible	Myles	GGAGAATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCGTT
Susceptible	CDC Frontier	GGAGAATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCGTT
Susceptible	CDC Corinne	GGAGAATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCGTT
Susceptible	CDC Luna	GGAGAATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCGTT
Resistant	ICCX860047-9	GGAGAATGATCGGAACCGATGTTTTTCAAGAAACCCCCATCGTT
Resistant	CDC Cory	GGAGAATGATCGGAACCGATGTTTTTCAAGAAACCCCCATCGTT
Resistant	CDC Alma	GGAGAATGATCGGAACCGATGTTTTTCAAGAAACCCCCATCGTT
		********** **********

B

Response to IMI herbicide	Genotype	Amino Acid Sequence (205)
Susceptible	Myles	MDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILE
Susceptible	CDC Frontier	MDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILE
Susceptible	CDC Corinne	MDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILE
Susceptible	CDC Luna	MDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILE
Resistant	ICCX860047-9	MDSIPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILE
Resistant	CDC Cory	MDSIPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILE
Resistant	CDC Alma	MDSIPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILE

C

Species and Genotype	Amino Acid Sequence (197, 205)
Arabidopsis thaliana	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMD
Medicago truncatula (SU-S)	MDSVPLIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILD
Medicago littoralis (SU-R)	MDSVPLIAITGQVLRRMIGTDAFQETPIVEVTRSITKHNYLILD
C. arietinum IMI-S consensus	MDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILE
C. arietinum IMI-R consensus	MDSIPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILE

Figure 3.1: Nucleotide and amino acid sequence alignments, susceptible genotype highlighted in yellow, resistant genotype highlighted in blue A) nucleotide alignment across seven chickpea (*Cicer arietinum*) genotypes, B) amino acid alignment across seven chickpea (*Cicer arietinum*) genotypes, C) amino acid alignment of chickpea and model plant *AHAS1* sequences. (Alignment based off of *Arabidopsis thaliana* NCBI reference NM_114714.2)

3.1.2. KASP SNP Genotyping

In total, 64 out of 70 RILs plus parental lines successfully yielded KASP SNP genotyping data. The population segregated into two clusters except for two lines (undefined data points in Figure 3.2). The parental lines each fell into different clusters. Based on clustering and parental florescence data, SNP genotypes were called as A = parental type CDC 512-51 (IMI susceptible; red) and B = parental type ICCX860047-1 (IMI resistant; blue). The SNP genotyping data was then compared to the herbicide rating data (Appendix 6). The KASP SNP genotyping platform accurately predicted herbicide response of 63/64 (98.4%) RILs. One line (#86) was inaccurately predicted.

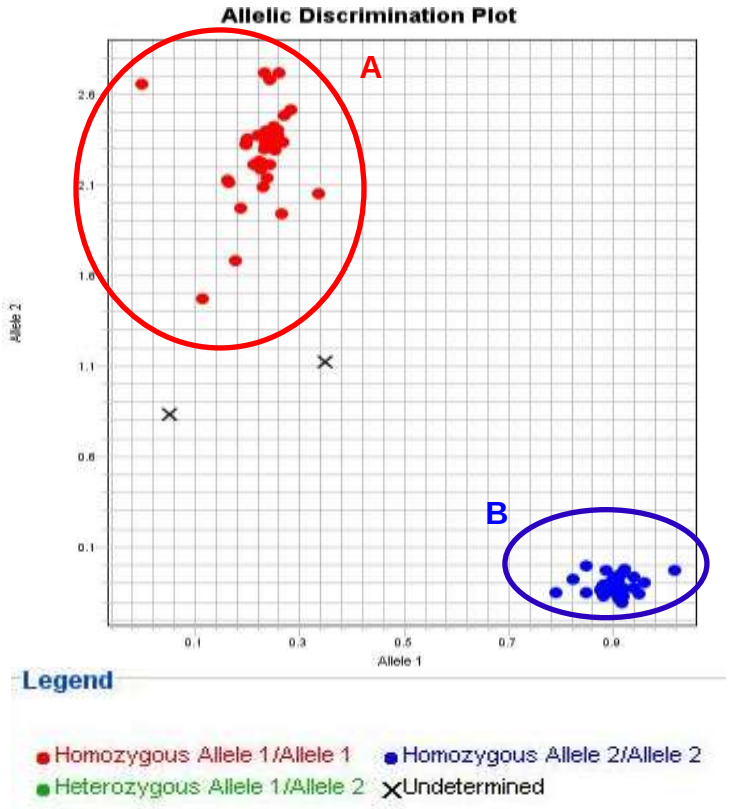

Figure 3.2: KASP SNP genotyping allelic discrimination plot of $F_{7:8}$ CDC 512-51 x ICCX 860047-1 population segregating for resistance to IMI herbicide. (Alignment based off of *Arabidopsis thaliana* NCBI reference NM_114714.2) A = parental type CDC 512-51 (IMI susceptible; red) and B = parental type ICCX860047-1 (IMI resistant; blue)

3.2. Component 2: Synteny of *AHAS* Gene

3.2.1. Lentil *AHAS* Sequencing

Lentil *AHAS1* sequence was generated by direct sequencing of PCR products. The sequence was included in the synteny analysis. Sequence alignment demonstrated that a mutation consistent with IMI herbicide resistance also occurs at the same location as chickpea, C675→T point mutation resulting in an Ala205→Val amino acid substitution (Figure 3.3). Full lentil *AHAS* sequences may be found in Appendix 3.

A

Species	Response to IMI herbicide	Genotype	Nucleotide Sequence (675 bp)
C. arietinum	Susceptible	CDC Frontier	AATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCG
C. arietinum	Resistant	ICCX860047-9	AATGATCGGAACCGATGTTTTTCAAGAAACCCCCATCG
L. culinaris	Susceptible	CDC Redberry	AATGATTGGAACTGATGCTTTTCAAGAAACCCCAATCG
L. culinaris	Resistant	CDC Impact	AATGATTGGAACTGATGTTTTTCAAGAAACCCCAATCG
			****** ***** **** *************** ****

B

Species	Response to IMI herbicide	Genotype	Amino Acid Sequence (205)
C. arietinum	Susceptible	CDC Frontier	IPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYL
C. arietinum	Resistant	ICCX860047-9	IPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYL
L. culinaris	Susceptible	CDC Redberry	IPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYL
L. culinaris	Resistant	CDC Impact	IPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYL

Figure 3.3. *AHAS1* Nucleotide and Amino acid sequence alignment of chickpea and lentil. (Alignment based on *Arabidopsis thaliana* NCBI reference NM_114714.2)

3.2.2. Cluster Analysis

The *Cicer arietinum* IMI-S consensus and IMI-R consensus were used as queries to search the BLAST® NCBI database to identify homologous *AHAS* sequences from other plant species. Table 3.1 shows the query results. Because chickpea *AHAS* has high similarity with other known *AHAS* genes, this suggests the chickpea sequences identified in this study in fact belong to the *AHAS* gene.

Table 3.1. NCBI BLAST® query results of searching *Cicer arietinum AHAS1* sequence

Species	NCBI Blast Accession Number	E-value[1]	% Identity
PREDICTED: Cicer arietinum acetolactate synthase 2, chloroplastic-like (LOC1015026	XM_004501646.1	0.0	99%
Medicago truncatula DNA sequence from clone MTH2-34D24 on chromosome 3, complete sequence	CT010521.4	0.0	85%
Medicago truncatula Acetolactate synthase (MTR_3g099190) mRNA, complete cds	XM_003602758.1	0.0	86%
Medicago truncatula acetolactate synthase (ALS1) gene, complete cds	EU292215.1	0.0	86%
Medicago littoralis cultivar Herald acetolactate synthase (ALS1) gene, complete cds	EU292214.1	0.0	86%
Medicago littoralis cultivar Angel acetolactate synthase (ALS1) gene, complete cds	EU292213.1	0.0	86%
Medicago truncatula cultivar Caliph acetolactate synthase (ALS1) gene, complete cds	EU292216.1	0.0	86%
Medicago truncatula Acetolactate synthase (MTR_2g027220) mRNA, complete cds	XM_003594271.1	0.0	83%
Medicago truncatula clone mth2-12p22, complete sequence	AC141866.11	0.0	83%
Lotus japonicus cDNA, clone: LjFL3-076-BG04, HTC	AK339751.1	0.0	81%
Lotus japonicus cDNA, clone: LjFL3-050-AA10, HTC	AK339673.1	0.0	81%
PREDICTED: *Cicer arietinum* acetolactate synthase 1, chloroplastic-like (LOC101513951), mRNA	XM_004485696.1	0.0	81%
PREDICTED: Glycine max acetolactate synthase 3, chloroplastic-like (LOC100798203), mRNA	XM_003528058.1	0.0	80%
PREDICTED: Glycine max acetolactate synthase 1, chloroplastic-like (LOC100782250)	XR_136475.1	0.0	79%

[1] E-value of 0.0 indicates an error rate less than 0.01%

Sequences representative of both *AHAS1* and *AHAS2* were used in cluster analysis and phylogenetic tree construction (Figure 3.4). *AHAS1* and *AHAS2* across pulse genera (*Cicer, Lens, Gycine, Lotus* and *Medicago*) cluster independently. In Figure 3.4, the *AHAS1* cluster is indicated by Cluster A and the *AHAS2* cluster is indicated by Cluster B. *AHAS* genes from other plant species; *Helianthus annuus, Sinapis arvensis* and *Brassica napus*, clustered independently (Cluster C, D and E, respectively).

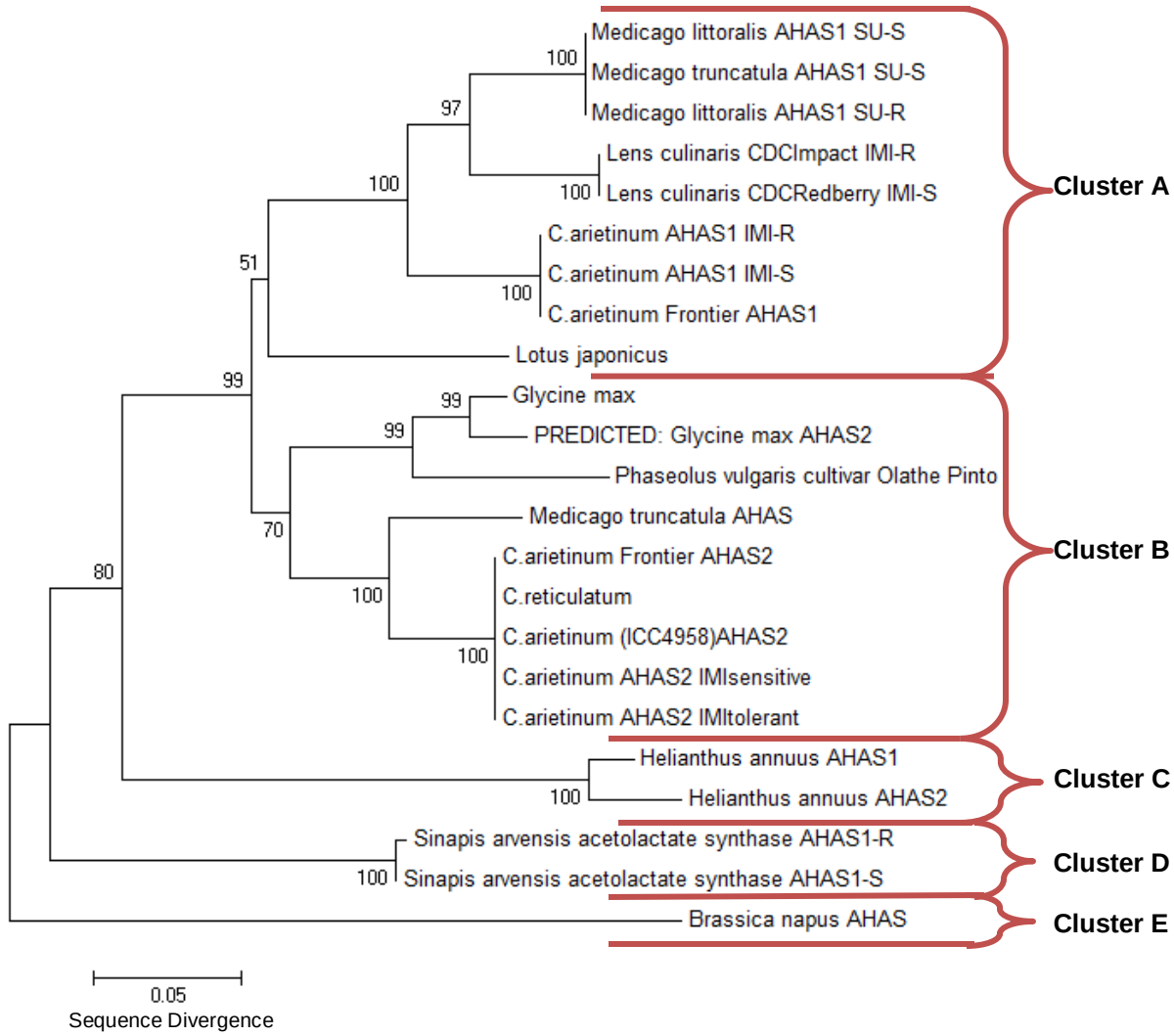

Figure 3.4. Phylogenetic tree of *AHAS1* and *AHAS2* in multiple plant species, using Neighbour-Joining method and bootstrap confidence levels (bootstrap replications = 500). Bootstrap confidence intervals are placed at each node and are expressed as a percentage.

3.3. Component 3: Molecular Mapping of *AHAS1* Gene

3.3.1. Molecular Mapping

The chickpea 1536 Illumina GoldenGate® SNP genotyping platform was used to develop a molecular map of the segregating population CDC 512-51 (IMI susceptible) x ICCX860047-9 (IMI resistant) (Figure 3.5) and confirm the location of the locus for IMI resistance. This map was then aligned with the ICCV96029 x CDC Frontier consensus reference map. Linkage group numbers correspond to chromosome numbers of the draft chickpea genome sequence (Varshney *et al.* 2013). Out of 1536 SNP markers, 530 were polymorphic between CDC 512-51 and ICCX860047-9 with 507 markers used to create the linkage map. These linkage groups correspond to the first seven ICCV96029 x CDC Frontier chromosomes (only the eighth chromosome is not accounted for)(Varshney *et al.* 2013). Two linkage groups did not correspond to the ICCV96029xCDC Frontier chromosome map most likely due to inadequate marker data. Mapping information is summarized in Table 3.2. The *AHAS* gene was linked to two SNP markers on chromosome 5; Cav1sc310.1p304295 (6.6 cM) and Cav1sc1.1p4940145 (3.8 cM) being the most closely linked (Figure 3.6). Other linkage groups are available in Appendix 7. The Cav1sc1.1p4940145 SNP marker was previously mapped to chromosome 5 in the ICCV96029xCDC Frontier chromosome map.

Figure 3.5: RIL parents' response to 1x rate of imidazolinone herbicide of Solo® (imazamox) at a rate of 20.22 g a.i. ha^{-1} (11.7 g. Solo®/acre), A) ICCX860047-9 IMI resistant, B) CDC 512-51 IMI susceptible

Table 3.2: Comparison of CDC512-51 x ICCX860047-9 RIL chickpea genetic map with ICCV96029xCDC Frontier consensus map

Chromosome #	Total cM CDC512-51 x ICCX860047-9 RIL		Total cM ICCV96029 x CDC Frontier	
	Map Size (cM)	# SNPs	Map Size (cM)	# SNPs
1	27.0	16	167.879	116
2	15.0	6	134.200	97
3	Map 1 = 7.4 Map 2 = 3.1	21 7	181.432	106
4	133.7	238	258.979	237
5	10.4	2	93.318	71
6	Map 1 = 1.4 Map 2 = 22.8	12 22	60.100	87
7	Map 1 = 39.0 Map 2 = 40.3	31 75	183.649	136

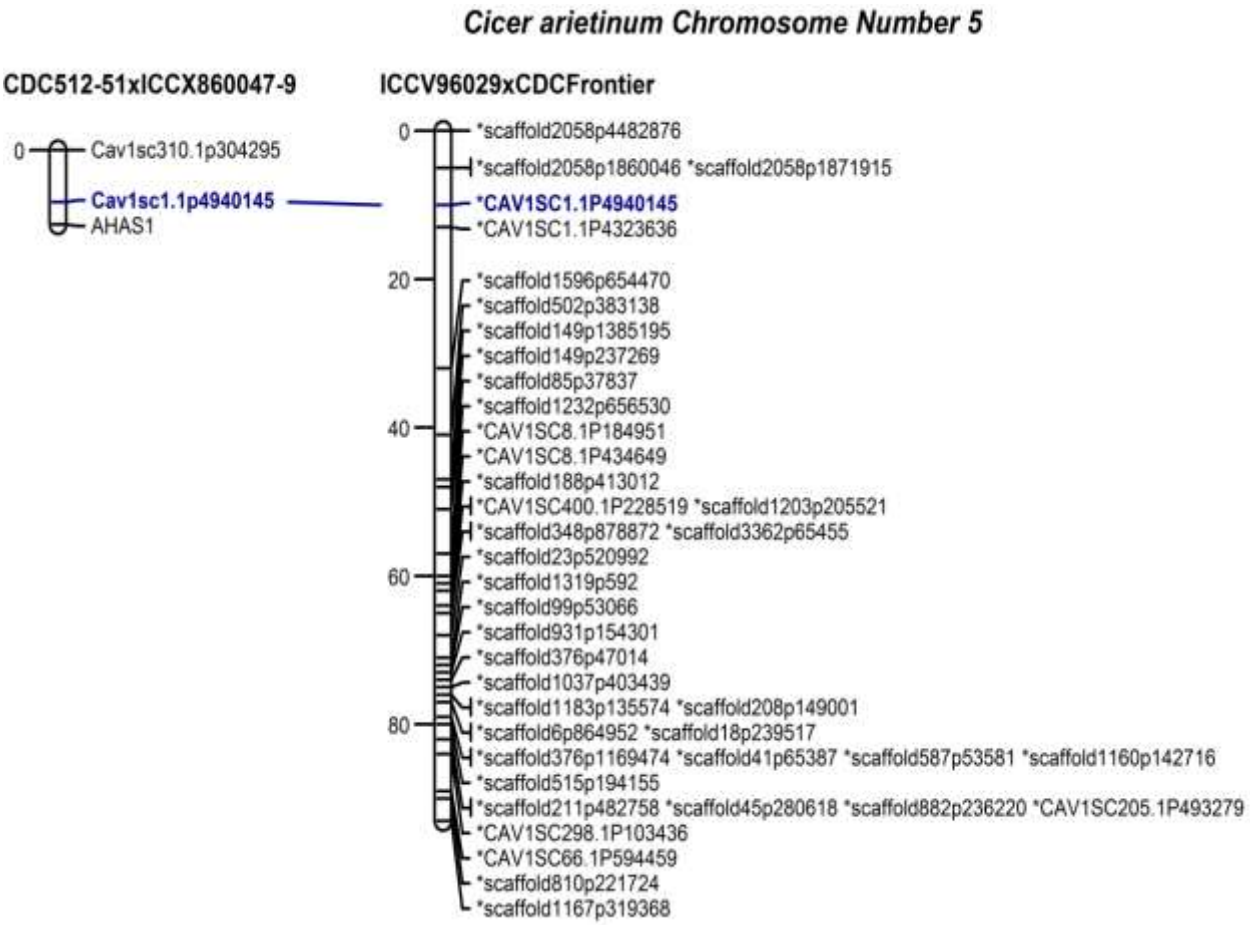

Figure 3.6: Comparison of *Cicer arietinum* Chromosome 5 of CDC512-51 x ICCX860047-9 RIL population segregating for IMI resistance to ICCV96029 x CDC Frontier consensus map (genetic distance in Kosambi cM).

3.3.2. Inheritance

The $F_{7:8}$ RIL population was derived from the cross of CDC512-51 (IMI susceptible) x ICCX 860047-9 (IMI resistant) segregated for herbicide resistance. If the $F_{7:8}$ generation (~98% homozygous) segregated following a single gene model, the expected ratio would be 50% IMI susceptible: 50% IMI resistant. Out of a total of 70 RILs, 40 lines were susceptible and 30 lines were resistant. The null hypothesis of this chi-square analysis is that segregation of this population is 50% resistant: 50% susceptible. The null hypothesis is accepted because the

calculated critical χ^2 value (1.429) is less than the critical χ^2 value (3.841) and the level of significance ($\alpha = 0.05$) is less than the p-value (0.232) (Table 3.3). Chi-square analysis suggests that IMI resistance in chickpea follows a single gene model and is inherited either as a dominant or semi-dominant nature.

Table 3.3. Chi square statistics for CDC512-51 x ICCX 860047-9 population segregating for IMI herbicide resistance

Population	Generation	Number Susceptible Plants	Number Resistant Plants	Critical Value	χ^2 Value	*P* Value
CDC512-51 x ICCX 860047-9	F7:8	40	30	3.841	1.429	0.232

3.4. AHAS Enzyme Activity

Enzyme activity was studied using a colorimetric assay specifically designed to target AHAS (Singh and Shaner 1988). This assay yielded both a visual representation and quantitative absorbance data. The AHAS specific *in vitro* and *in vivo* colorimetric assays involved the conversion of the AHAS product acetolactate to acetoin then a pink coloring was produced by incubation with napthol and creatine. The degree of coloring is an indication of how much acetolactate was produced and approximate level of AHAS activity. Both Figure 3.7 and 3.8 show the results of this assay, with absorbance values and additional data in Appendix 8.

The *in vitro* study tested the null hypothesis that there is no significant difference of the acetoin production between the two genotypes across different IMI concentrations. A two factor ANOVA analysis was completed using acetolactate concentration converted from absorbance values using a standard curve. The results of ANOVA (Appendix 8, Table 6.2), indicated that IMI concentration and chickpea genotype significantly affected the acetolactate production. The *in vitro* study (Figure 3.7) showed increasing inhibition of AHAS enzyme in CDC Frontier as

IMI concentration increased. At a concentration of 8 µM IMI, acetolactate concentration was reduced to12.20 µM. At 8 µM IMI CDC Cory showed a maximum drop in acetolactate production to 76.95µM.

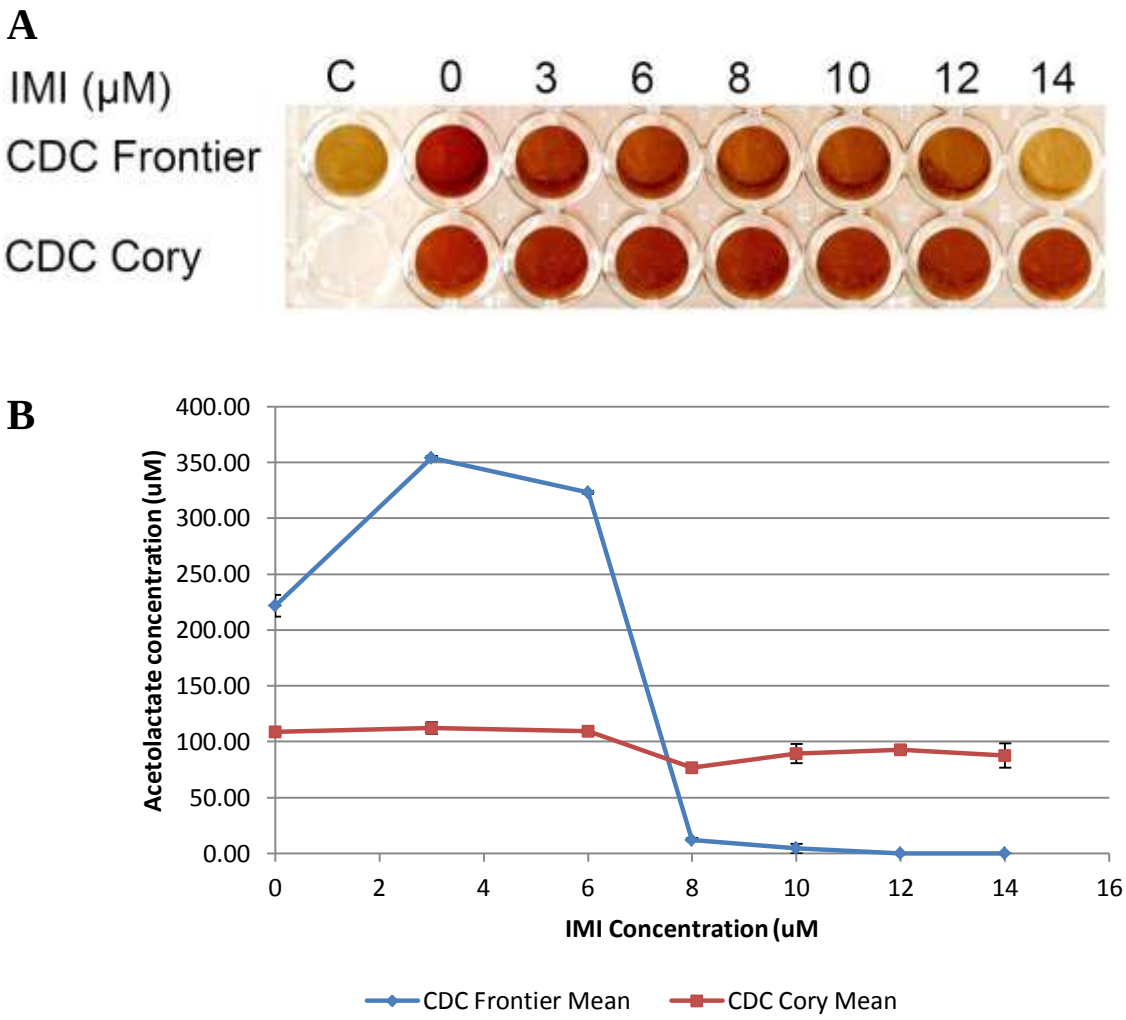

Figure 3.7. A) *In vitro* colorimetric assay for AHAS enzyme activity with increasing IMI concentration for CDC Frontier (IMI susceptible) and CDC Cory (IMI resistant), B) graphical representation of colorimetric assay (absorbance values converted to acetolactate concentration using a standard curve).

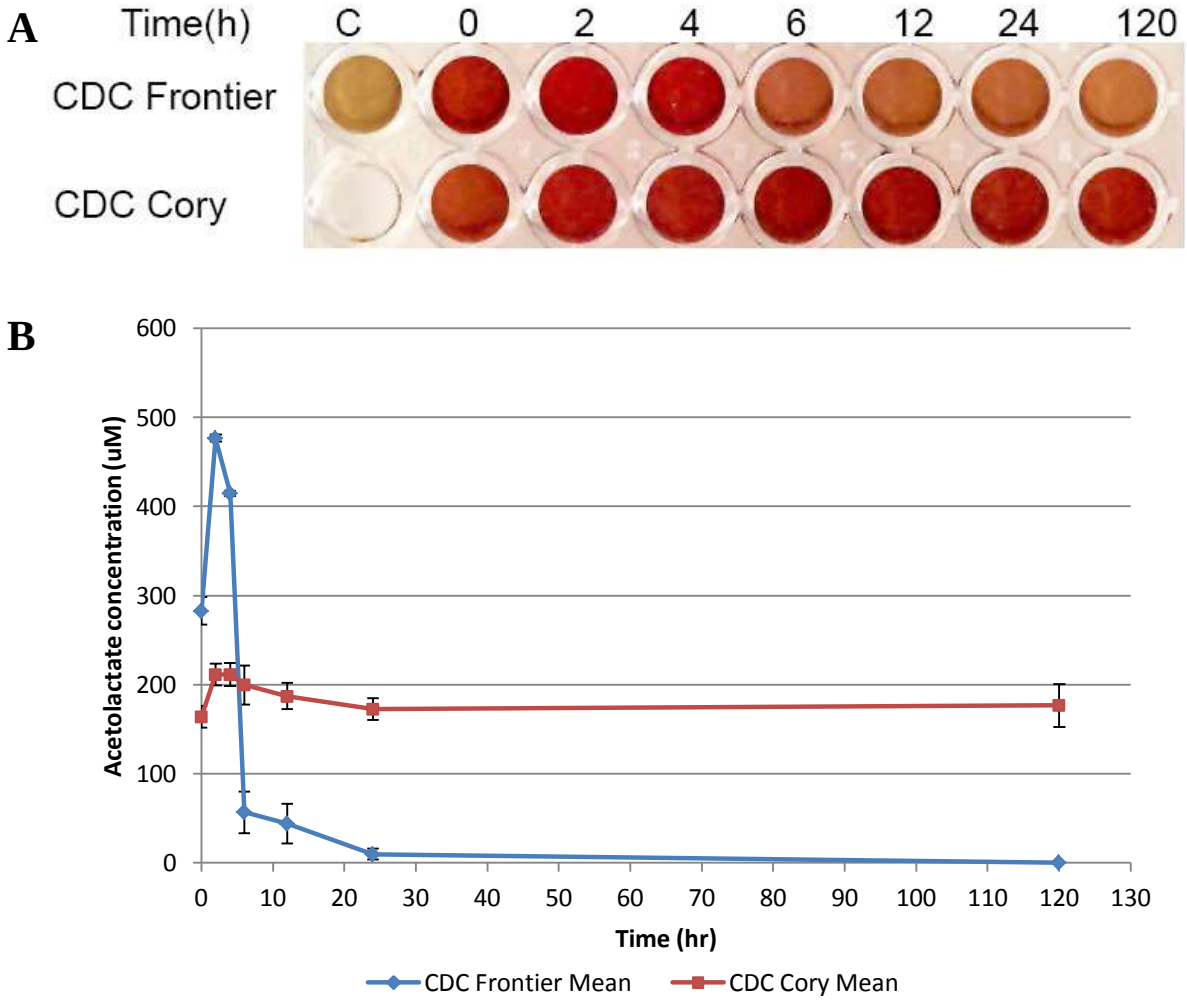

Figure 3.8. A) *In vivo* colorimetric assay for AHAS enzyme activity over time after treatment with IMI for CDC Frontier (IMI susceptible) and CDC Cory (IMI resistant), B) graphical representation of colorimetric assay (absorbance values converted to acetolactate concentration using a standard curve).

The results of *in vivo* assay showed similar trend (Figure 3.8, Appendix 8). CDC Frontier (IMI susceptible) acetolactate production started to decline after 4 hr of IMI treatment, decreasing to 9.23 µM at 24hr. However, CDC Cory (IMI resistant) acetolactate production decreased to only 172.87 µM (Appendix 8, Table 6.1). Both genotypes did show an initial increase in acetolactate production; 2 – 4hr for CDC Frontier, and 2–12 hr for CDC Cory. A separate ANOVA was carried out at each time interval (Appendix 8, Table 6.3). The *in vivo* tested the null hypothesis that there is no significant difference over times between the IMI resistant and the IMI susceptible genotypes for the acetolactate production in response to IMI treatment. Based on the ANOVA results, at each time interval, genotype has a significant effect on the acetolactate production ($P < 0.05$).

In summary, both *in vitro* and *in vivo* assays CDC Frontier showed increasing AHAS inhibition over time and increasing IMI concentrations; however CDC Cory did not show increasing AHAS inhibition over time, it only showed a slight inhibition at a concentration of 8 µM IMI.

4. Discussion

The availability of varieties with resistance to Group 2 herbicides could expand the spectrum of broadleaf weed management in chickpea, a challenge presently faced by Western Canadian chickpea growers. Through conventional breeding, IMI resistant chickpea varieties CDC Cory and CDC Alma (herbicide registration pending) have been developed at the Crop Development Centre (College of Agriculture and Bioresources, University of Saskatchewan). In this study, the *AHAS* gene responsible for IMI resistance in chickpea was sequenced and compared with its orthologs across different species. The *AHAS1* sequences were used to develop an allele specific (KASP) marker targeting the point mutation leading to IMI resistance. The availability of this marker could enhance the selection process in the breeding program to develop resistant varieties. A RIL population segregating for IMI resistance was used in molecular mapping to confirm the location of the resistance gene in the chickpea genome. The same population was also used to examine the accuracy of the KASP marker to discriminate the resistant lines and to examine the mode of inheritance of the resistance. Preliminary AHAS enzyme activity analysis using colorimetric assays confirmed the inhibition of the enzyme in the susceptible genotype as a result of IMI application. Together this information helps to better understand the role *AHAS* in Group 2 herbicide resistance in chickpea.

The *AHAS* gene has been extensively studied and reviewed in multiple plant species (Tan *et al.* 2005). Group 2 herbicides inhibit the *acetohydroxyacid synthase* gene (*AHAS*), and resistance is usually the result of a point mutation in the *AHAS* gene causing amino acid substitution in the AHAS enzyme (Tan *et al.* 2005; Beckie and Tardif 2012). Point mutations in *AHAS* have been linked to linked to varying degrees and spectrum of resistance to Group 2 herbicides (Tan *et al.* 2005). *AHAS* sequences from two pulse species, *Lens culinaris* (lentil) and *Cicer arietinum*

(chickpea), were generated and compared. In chickpea and lentil *AHAS*, a C→T mutation at nucleotide #675 resulting in an Ala205→Val substitution confers the resistance to IMI. In Redroot Pigweed (*Amaranthus retroflexus*) and Eastern Black Nightshade (*Solanum Ptychanthum*) an Ala205 substitution causes IMI resistance (McNaughton *et al.* 2005; Ashigh and Tardif 2007; Beckie and Tardif 2012). However, research in sunflower (*Helianthus annuus*) showed an Ala205 substitution resulted in IMI resistance with partial SU resistance. McCourt *et al.* (2006), did not identify Ala205 as a IMI binding site, so more structural analysis on the effect of Ala205 substitutions on herbicide binding may be needed. In *Medicago spp.* a Pro197→Leu resulted in SU resistance (Figure 4.1) (Oldach *et al.* 2008). A Pro197 substitution in the *AHAS* gene results in phenotype with varying levels of resistance to SU, PTB and TP (Haughn *et al.* 1988; Mourad and King 1992; Beckie and Tardif 2012), which is consistent with the findings of McCourt *et al*. (2006) that Pro197 is in direct contact with SU but indirectly with IMI. This suggests that specific Group 2 resistance (IMI, PTB, SCT, TP or SU) or cross resistance among them is associated with the mutation site(s) within the *AHAS* gene and may not be conserved across different species.

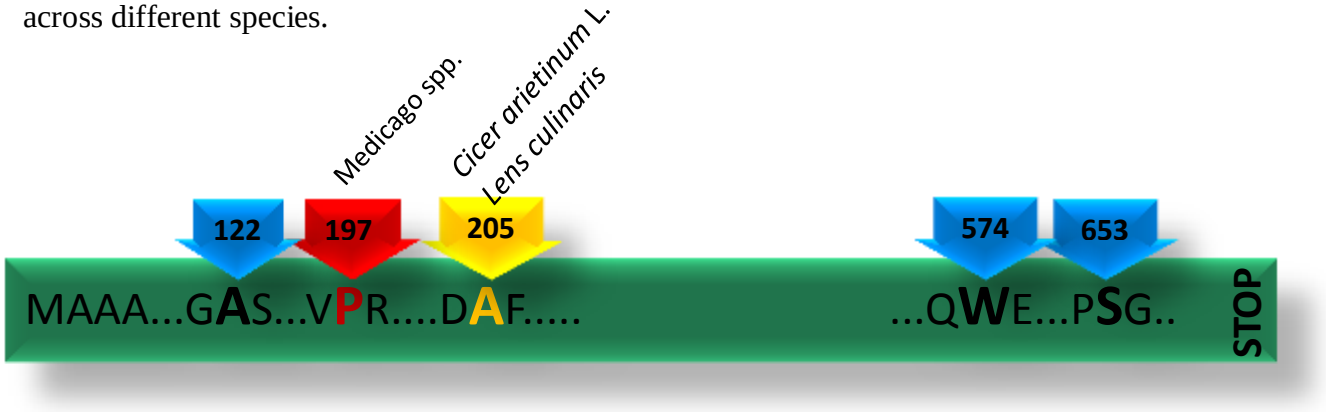

Figure 4.1.Common mutation sites in the *AHAS* gene that cause resistance to Group 2 herbicides across various plant species (amino acid numbering in arrows based on the *Arabidopsis thaliana* NCBI reference NM_114714.2) (Tan *et al.* 2005). Pulse species holding the specific mutation site causing Group 2 resistance is indicated above the mutation site arrow.

Depending on the species, there may be a single copy or multiple copies of *AHAS*. In *Arabidopsis thaliana*, only *CSR1* codes for AHAS (Haughn and Somerville 1986; Haughn *et al.* 1988; Manabe *et al.* 2007). However, some species have multiple *AHAS* genes. In *Brassica napus*, *AHAS1* and *AHAS2* are 85% similar and *AHAS1* and *AHAS3* are 98% similar (Rutledge *et al.* 1991). Rutledge *et al*. (1991) determined that each copy may have originated from each *Brassica napus* ancestor genome. RNase protection assays showed that *AHAS*1 and *AHAS3* were expressed in all plant tissues, but *AHAS2* was only expressed in mature ovule and immature seed tissue, meaning *AHAS2* may have a specific role in seed development in *Brassica napus* (Ouellet *et al.* 1992). Sunflower (*Helianthus annuus*) has three homologous *AHAS* genes, *AHAS1* and *AHAS2* are 92% identical, and *AHAS3* is only 72% identical to *AHAS1* and 73% similar to *AHAS2,* respectively (Kolkman *et al.* 2004). Sunflower *AHAS1* and *3* were predominantly expressed in leaf tissue, which is logical since AHAS is located in chloroplasts (Miflin 1974; Smith *et al.* 1989). Genetic characterization between gene resistances found on different wheat genomes has shown interesting results. Work by Hanson *et al*. (2006; 2007) shows differing levels of IMI resistance depending on number of resistance genes (i.e. staking resistance genes in different wheat genomes). Differences in recovery after IMI treatment and differences between whole plant biomass accumulation studies vs enzyme activity assays suggests other factors like ability to metabolize IMI, physiology at application and resistant gene expression may also affect the plant's ability to recover after IMI treatment (Hanson *et al.* 2006; Hanson *et al.* 2007). In chickpea, there are two homologous *AHAS* genes, *AHAS1* and *AHAS2.* Even though the nucleotides sequences are 81% (amino acid sequences 80%) similar, only a mutation in *AHAS1* confers IMI resistance. To date, the role of multiple *AHAS* genes in chickpea is unknown and additional research may be needed.

Chickpea *AHAS1*/*AHAS2* and lentil *AHAS1* sequences were compared to other known *AHAS* sequences by multiple alignment. Sequence alignment of the chickpea and lentil *AHAS1* revealed that the same point mutation is responsible for IMI resistance in chickpea and lentil, however this mutation is different than that observed in *Medicago spp*. (Figure 4.1, Appendix 4) (Oldach *et al.* 2008). When aligning the *Arabidopsis thaliana, Medicago spp., Lens culinaris, C. arietinum AHAS1* consensus amino acid sequences, some observations can be made (Appendix 4). A small portion of the 5' region of chickpea *AHAS* was not completely sequenced and 158 amino acid residues (5' region) are missing in *Lens culinaris* 'CDC Redberry' IMI-S. This may be due to higher variation in the 5' region of the *AHAS* gene resulting in poor amplification. Cluster analysis showed that *AHAS1* and *AHAS2* genes tend to cluster independently, except in non-legume species *Sinapis arvens, Helianthus annuus* and *Brassica napus*. Because *AHAS1* and *AHAS2* cluster independently between *Cicer spp* and *Medicago spp,* this implies that a specific mutation event in a legume ancestor gave rise to duplication of the *AHAS* gene in legume species. Additional sequencing of *AHAS2* in other pulse species may be needed to confirm this conclusion. For each *AHAS* gene, *Medicago spp.* and chickpea are grouped together (Figure 3.4). This is in agreement with findings of Varshney *et al*. (2013) where 89.7% chickpea proteins are similar to *Medicago truncatula*.

An allele specific marker (KASP) was developed and tested for its potential use in marker assisted selection (MAS) for IMI resistant chickpea. Efficient molecular markers for use in MAS should possess the following characteristics: polymorphic, differentiate between homozygous and heterozygous genotypes, have even distribution throughout the genome, have little GxE impacts, low cost, and have a straight-forward procedure and reproducible results (Kumar *et al.* 2009). Use of SNP markers have been reviewed and the main benefits include good distribution

throughout the genome, no electrophoresis required, low cost, reproducible results and potential for automation (Syvanen 2005; Xu and Crouch 2008). This study used the point mutation identified in section 3.1.1 to develop an allele specific SNP marker to screen a chickpea RIL population segregating for herbicide resistance. This marker accurately predicted phenotypic response to IMI herbicides in 63 out of 64 lines. To confirm marker efficiency and accuracy, larger segregating populations should be screened.

The chickpea 1536 Illumina GoldenGate® assay (Illumina, San Diego, CA USA; Fan *et al.* 2006) was used to construct a linkage map and determine the location of the *AHAS* gene. This assay is an automated, pre-optimized assay which can collect 96, 384, 768, 1536 SNP markers per sample. Once the platform has been specifically developed and optimized for a specific crop species, it can quickly produce a large amount of SNP data on a population. Previously, chickpea molecular mapping was done using SSR (simple sequence repeat) markers (Tar'an *et al.* 2007; Anbessa *et al.* 2009); however SNP markers are becoming more popular for genotyping and mapping applications. SNP markers may be used to construct molecular maps, study gene synteny or for trait screening (Li *et al.* 2008; Ganal *et al.* 2009; Hiremath *et al.* 2012; Mammadov *et al.* 2012). High-throughput SNP genotyping platforms have allowed for high density and detailed genetic maps to be developed in chickpea. Gaur *et al.* (2012) developed an Illumina GoldenGate® SNP genotyping platform for creating a detailed and comprehensive molecular map for chickpea using an interspecific cross of *Cicer arietinum* and *Cicer reticulatum*. This map consisted of 696 SNP markers in conjunction with other available marker data from SSRs, ITPs (intron targeted polymorphisms) and ESTs (expressed sequence tag) for linkage group construction. The recently published chickpea draft genome (Varshney *et al.* 2013) provides a reference to compare molecular maps with a physical map

This study used 1536 SNPs in the Illumina GoldenGate® genotyping platform developed and optimized at the National Center for Biotechnology Information (Saskatoon SK, Canada) and was used to map the location of the *AHAS* gene in chickpea. The map from the CDC 512-51 x ICCX860047-9 RIL population covered seven out of the eight chickpea chromosomes with the *AHAS* gene located on chromosome 5. IMI response segregated as a single gene and was linked to two SNP markers namely Cav1sc310.1p304295 and Cav1sc1.1p4940145. However this linkage group only consisted of these two markers. Low marker coverage may be due to small sample size (<70 RIL lines), lack of recombination or variation (besides IMI resistance) between the parents (CDC 512-51 x ICCX860047-9) where both parents are Desi type.

A colorimetric assay to show differences in AHAS enzyme activity in chickpea treated with IMI by measuring the amount of acetolactate produced. It has been determined that Group 2 herbicides inhibit the AHAS enzyme. The *in vitro* study also showed that with increasing IMI concentrations, CDC Frontier AHAS became completely inhibited at 12 µM IMI, but CDC Cory's acetolactate production is only reduced to a maximum of 76.95 µM at 8µM IMI. The *in vivo* assay showed that CDC Frontier (IMI susceptible) treated with IMI caused a large decrease in acetolactate production (0 µM at 5 days after IMI treatment) but CDC Cory (IMI resistant) showed only slight inhibition (172.87 µM 24 hr after IMI treatment). These findings are in agreement with the early research by Muhitch *et al.* (1987), and reviewed by Duggleby and Pang (2000) showing that AHAS is the target of IMI herbicides and inhibition is relatively slow. This information together with *AHAS* sequence analysis, suggests that a point mutation in chickpea *AHAS1* (identified in section 3.1.1) may prevent IMI herbicides from binding and inhibiting the AHAS enzyme activity, which is consistent with previous research (Tan *et al.* 2005; Lee *et al.* 2011). In *Medicago spp*. barley and sunflower (*Helianthus annuus*) AHAS expression was

significantly decreased in susceptible genotypes (30 – 40% in *Medicago*) (Oldach *et al.* 2008; Lee *et al.* 2011; Breccia *et al.* 2013). Additional studies using qPCR may be used to show the effects of IMI on chickpea AHAS expression. The AHAS catalytic structure holds the Group 2 herbicide binding site (Pang *et al.* 2002; McCourt *et al.* 2006). Both IMI and SU herbicides bind to the channel leading to the AHAS active site, blocking the AHAS substrate access to the active site (Pang *et al.* 2002; McCourt *et al.* 2006). Even though Ala205 is a key residue in Group 2 herbicide resistance (Tan *et al.* 2005), this site was not identified in enzyme structural analysis (McCourt *et al.* 2006; Duggleby *et al.* 2008). *Arabidopsis thaliana* AHAS analysis conducted by McCourt et al. (2006) used imazaquin, where other IMI chemistries may have other binding sites within AHAS. More AHAS structural analysis while in complex with Group 2 herbicides would be required to identify all key AHAS herbicide binding sites. Lastly, an initial increase in acetolactate production in both CDC Frontier and CDC Cory was observed. This may be the plant's initial reaction to IMI treatment; however additional data may be needed to confirm this phenomenon.

Summary and Conclusions

This study genetically characterized imidazolinone resistance in cultivated chickpea (*Cicer arietinum*). Molecular mapping, synteny/cluster analysis and some protein assays were carried out to gain further information on the role of AHAS in chickpea. All of this information together gives insight to the molecular mechanisms chickpea has for herbicide resistance, and also provides valuable information that could be used to more efficiently breed IMI resistant chickpea varieties.

The main conclusions of this research are as follows: Two homologous *AHAS* genes are present in the chickpea genome and a point mutation in the *AHAS1* gene results in IMI resistance. After sequencing lentil *AHAS1*, it was determined that the same point mutation as chickpea also causes IMI resistance in lentil. Using this information, an efficient KASP SNP marker was developed for screening chickpea populations for IMI resistance. Segregation analysis using an IMI treated RIL population showed that IMI resistance is controlled by a single gene and is either semi dominant or dominantly inherited. Using the Illumina GoldenGate® SNP genotyping assay, IMI resistance was mapped to chromosome 5 similar to the position in pseudomolecules of CDC Frontier. When comparing *AHAS* sequences across other pulse species through cluster analysis, *AHAS1* and *AHAS2* sequences cluster separately.

This study shows that even though the *AHAS* genes are homologous across different plant species, the mutation site is not necessarily conserved. Group 2 resistance or cross resistance among herbicides is associated with the mutation site(s) within the *AHAS* gene and may not be conserved across different species.

Future studies should focus on expanding the understanding of both the AHAS gene and enzyme. There is a gap in knowledge regarding multiple homologous *AHAS* genes within a species genome. Understanding the role of the genes may yield understanding in plant response to AHAS inhibiting herbicides. In addition, more work in AHAS structure and function when in complex with Group 2 herbicides would also add to understanding of herbicide response.

Understanding the genetic mechanism of herbicide resistance may increase efficiency of crop variety development, benefitting Saskatchewan chickpea farmers. Developing varieties with more herbicide group options would provide more weed management options for chickpea farmers. Even though there are IMI resistant weeds identified, IMI applications in chickpea crops may help control other problem broadleaf weeds. Information from this study adds to genetic understanding of the trait, but also could be used in efficient selection of progeny in the chickpea breeding program.

References

Agriculture and Agri-food Canada (2008) Crop profile for chickpea in Canada.

Anbessa Y., Taran B., Warkentin T.D., Tullu A., Vandenberg A. (2009) Genetic analyses and conservation of QTL for *ascochyta* blight resistance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 119:757-765

Ashigh J., Tardif F.J. (2007) An Ala205val substitution in acetohydroxyacid synthase of Eastern Black Nightshade (*Solanum Ptychanthum*) reduces sensitivity to herbicides and feedback inhibition. Weed Sci 55:558-565

Baker C.J., Saxton K.E., Ritchie W.R., Chamen W.C.T., Reicosky D.C., Ribeiro M.F.S., Justice S.E., Hobbs P.R. (1996) No-tillage seeding in conservation agriculture. CAB International, Wallingford, UK

Banniza S., Armstrong-Cho C.L., Gan Y., Chongo G. (2011) Evaluation of fungicide efficacy and application frequency for the control of *ascochyta* blight in chickpea. Can J of Plant Pathol 33:135-149

Beckie H.J., Tardif F.J. (2012) Herbicide cross resistance in weeds. Crop Prot 35:15-28

Bernasconi P., Woodworth A.R., Rosen B.A., Subramnian M.V., Siehl D.L. (1995) A naturally occurring point mutation confers broad range tolerance to herbicides that target Acetolactate Synthase. J Biol Chem 270:5

Breccia G., Vega T., Felitti S.A., Picardi L., Nestares G. (2013) Differential expression of acetohydroxyacid synthase genes in sunflower plantlets and its response to imazapyr herbicide. Plant Sci 208:28-33

Buhariwalla H.K., Jayashree B., Eshwar K., Crouch J.H. (2005) Development of ESTs from chickpea roots and their use in diversity analysis of the Cicer genus. BMC Plant Biol 5:16

Choudhary P., Khanna S.M., Jain P.K., Bharadwaj C., Kumar J., Lakhera P.C., Srinivasan R. (2013) Molecular characterization of primary gene pool of chickpea based on ISSR markers. Biochem Genet 51:306-322

Christoffers M.J., Nandula V.K., Howatt K.A., Wehking T.R. (2006) Target-site resistance to acetolactate synthase inhibitors in wild mustard (*Sinapis arvensis*). Weed Sci 53:191-197

Cobb A.H., Read J.P.H. (2010) Herbicides and Plant Physiology. 2nd edn. John Wiley and Sons, Oxford, UK

Cutforth H.W., McGinn S.M., McPhee K.E., Miller P.R. (2007) Adaptation of Pulse Crops to the Changing Climate of the Northern Great Plains. Agron J 99:1684

de Givry S., Bouchez M., Chabrier P., Milan D., Schiex T. (2005) CARHTA GENE: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics 21:1703-1704

Doyle J.J., Doyle J.L. (1987) CTAB DNA extraction in plants. Phytochem Bull 19:11-15

Duggleby R.G., McCourt J.A., Guddat L.W. (2008) Structure and mechanism of inhibition of plant acetohydroxyacid synthase. Plant Physiol Biochem 46:309-324

Duggleby R.G., Pang S.S. (2000) Acetohydroxyacid Synthase. J of Biochem and Mol Biol 33:1-36

Duggleby R.G., Pang S.S., Yu H., Guddat L.W. (2003) Systematic characterization of mutations in yeast acetohydroxyacid synthase. Interpretation of herbicide-resistance data. European J of Biochem 270:2895-2904

Fan J.B., Chee M.S., Gunderson K.L. (2006) Highly parallel genomic assays. Nat Rev Genet 7:632-644

FAOSTAT (2013). http://faostat.fao.org/. Accessed 2 April 2013

Felton W.L., Haigh B.M., Harden S. (2004) Comparing weed competition in chickpea, fababean, canola and wheat.pdf. In: 14th Australian Weeds Conference, Tamworth, New South Wales, Australia. Weed Society of New South Wales,

Foley M.F., Bauman T.T. (1995) Absorption, translocation, and metabolism of imazethapyr in Common Ragweed (*Ambrosia artemisiifolia*) and Giant Ragweed (*Ambrosia trifida*). Weed Sci 43:572-577

Ganal M.W., Altmann T., Roder M.S. (2009) SNP identification in crop plants. Curr Opin Plant Biol 12:211-217

Garg R., Patel R.K., Jhanwar S., Priya P., Bhattacharjee A., Yadav G., Bhatia S., Chattopadhyay D., Tyagi A.K., Jain M. (2011) Gene discovery and tissue-specific transcriptome analysis in chickpea with massively parallel pyrosequencing and web resource development. Plant Physiol 156:1661-1678

Gaur R., Azam S., Jeena G., Khan A.W., Choudhary S., Jain M., Yadav G., Tyagi A.K., Chattopadhyay D., Bhatia S. (2012) High-throughput SNP discovery and genotyping for constructing a saturated linkage map of chickpea (*Cicer arietinum* L.). DNA Res 19:357-373

Geisel B.G.L., Schoenau J.J., Holm F.A., Johnson E.N. (2008) Interactions of ALS-inhibiting herbicide residues in three prairie soils. Weed Sci 56:624-627

Han H., Yu Q., Purba E., Li M., Walsh M., Friesen S., Powles S.B. (2012) A novel amino acid substitution Ala-122-Tyr in ALS confers high-level and broad resistance across ALS-inhibiting herbicides. Pest Manag Sci 68:1164-1170

Hanson B.D., Fandrich L., Shaner D.L., Westra P., Nissen S.J. (2007) Recovery of Imidazolinone-Resistant Hard Red Wheat Lines Following Imazamox Application. Crop Science 47:2058

Hanson B.D., Shaner D.L., Westra P., Nissen S.J. (2006) Response of selected Hard Red Wheat lines to Imazamox as affected by number and location of resistance genes, parental background, and growth habit. Crop Sci 46:1206-1211

Haughn G.H., Somerville C.R. (1990) A Mutation Causing Imidazolinone Resistance Maps to the Csrl Locus of *Arabidopsis thaliana*. Plant Physiol 92:1081-1085

Haughn G.W., Smith J., Mazur B., Somerville C. (1988) Transformation with a mutant Arabidopsis acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Molecular and General Genetics 211:266-271

Haughn G.W., Somerville C. (1986) Sulfonylurea-resistant mutants of Arabidopsis thaliana. Mol Gen Genet 204:430-434

Heap I. (2013) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed 2 May 2013

Heap J. (2000) Increasing Medicago resistance to soil residues of ALS-inhibiting herbicides. University of Adelaide, Adelaide, Australia

Hiremath P.J., Kumar A., Penmetsa R.V., Farmer A., Schlueter J.A., Chamarthi S.K., Whaley A.M., Carrasquilla-Garcia N., Gaur P.M., Upadhyaya H.D., Kavi Kishor P.B., Shah T.M., Cook D.R., Varshney R.K. (2012) Large-scale development of cost-effective SNP marker assays for diversity assessment and genetic mapping in chickpea and comparative mapping in legumes. Plant Biotechnol J 10:716-732

Iruela M., Rubio J., Cubero J.I., Gil J., Millan T. (2002) Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. Theor Appl Genet 104:643-651

Kantar F., Elkoca E., Zengin H. (1999) Chemical and Agronomical Weed Control in Chickpea (*Cicer arietinum* L. cv. Aziziye-94). Tr J of Agric and For 23:631-635

Kashiwagi J., Krishnamurthy L., Upadhyaya H.D., Krishna H., Chandra S., Vadez V., Serraj R. (2006) Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213-222

Kolkman J.M., Slabaugh M.B., Bruniard J.M., Berry S., Bushman B.S., Olungu C., Maes N., Abratti G., Zambelli A., Miller J.F., Leon A., Knapp S.J. (2004) Acetohydroxyacid synthase mutations conferring resistance to imidazolinone or sulfonylurea herbicides in sunflower. Theor Appl Genet 109:1147-1159

Kosambi D.D. (1943) The Estimation of Map Distances From Recombination Values. Annals of Eugenics 12:172-175

Kumar P., Gupta V.K., Misra A.K., Modi D.R., Pandey B.K. (2009) Potential of molecular markers in plant biotechnology. Plant Omics J 2:141-162

Kupicha F.K. (1977) The delimitation of the tribe Vicieae (Legurninosae) and the relationships of *Cicer* L Bot J of the Linnean Soc 74:131-162

Ladizinsky G., Adler A. (1976) The origin of chickpea *Cicer arietinum* L. Euphytica 25:211-217

Lavin M., Herendeen P.S., Wojciechowski M.F. (2005) Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. Syst Biol 54:575-594

Lee H., Rustgi S., Kumar N., Burke I., Yenish J.P., Gill K.S., von Wettstein D., Ullrich S.E. (2011) Single nucleotide mutation in the barley acetohydroxy acid synthase (AHAS) gene confers resistance to imidazolinone herbicides. Proc Natl Acad Sci U S A 108:8909-8913

Lee T.U., Duggleby R.G. (2001) Identification of the regulatory subunit of *Arabidopsis thaliana* acetohydroxyacid synthase and reconstitution with its catalytic subunit. Biochemistry 40:6836-6844

Lee Y.T., Chang A.K., Duggleby R.G. (1999) Efect of mutagenesis at serine 653 of Arabidopsis thaliana acetohydroxyacid synthase on the sensitivity to imidazolinone and sulfonylurea herbicides. FEBS Letters 452:341-345

Lee Y.T., Duggleby R.G. (2002) Regulatory interactions in *Arabidopsis thaliana* acetohydroxyacid synthase. FEBS Letters 512:180^184

Leisso R.S., Miller P.R., Burrows M.E. (2009) The influence of biological and fungicidal seed treatments on chickpea (*Cicer arietinum*) damping off. Can J of Plant Pathol 31:38 - 46

Li D., Barclay I., Jose K., Stefanova K., Appels R. (2008) A mutation at the Ala122 position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: improved resistance to imidazolinone and a faster assay for marker assisted selection. Molecular Breeding 22:217-225

Maiti R.K., Wesche-Ebeling P. (2001) Vegetative and reproductive growth and productivity. . In: Maiti RK, Wesche-Ebeling P (eds.) Advances in Chickpea Science. Science Publishers, Inc., Enfield, NH, USA, pp 67-104

Mallory-Smith C.A., Retzinger E.J. (2003) Revised classification of herbicides by site of action for weed resistance management strategies. Weed Technol 17:605-619

Mammadov J., Aggarwal R., Buyyarapu R., Kumpatla S. (2012) SNP markers and their impact on plant breeding. Int J Plant Genomics 2012:728398

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

McCourt J.A., Pang S.S., King-Scott J., Guddat L.W., Duggleby R.G. (2006) Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. Proc Natl Acad Sci U S A 103:569-573

McKay K., Miller P., Jenks B., Riesselman J., Neill K., Buschena D., Bussan A.J. (2002) Growing Chickpea in the Northern Great Plains. North Dakota State University Extension Bulletin A-1236, Fargo, North Dakota

McNaughton K.E., Letarte J., A L.E., Tardif F.J. (2005) Mutations in ALS Confer Herbicide Resistance in Redroot Pigweed (*Amaranthus retroflexus*)and Powell Amaranth (*Amaranthus powellii*). Weed Science 53:17-22

Miflin (1974) The location of nitrite reductase and other enzymes related to amino acid biosynthesis in the plastids of root and leaves. Plant Physiol 54:550-555

Moreno M.T., Cubero J.I. (1978) Variation in *Cicer arietinum* L. Euphytica 27:465-485

Moss G.P.(2013)EC 2 Transferases INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY. http://www.chem.qmul.ac.uk/iubmb/.

Mourad G., King J. (1992) Effect of four classes of herbicides on growth and acetolactate-synthase activity in several variants of *Arabidopsis thaliana*. Planta 188:491-497

Muhitch M.J., Shaner D.L., Stidham M.A. (1987) Imidazolinones and acetohydroxyacid synthase from higher plants. Plant Physiol 83:451-456

Newhouse K.E., Smith W.A., Starrett M.A., Schaefer T.J., Singh B.K. (1992) Tolerance to imidazolinone herbicides in wheat. Plant Physiol 100:882-886

Nguyen T.T., Taylor P.W.J., Redden R.J., Ford R. (2004) Genetic diversity estimates in Cicer using AFLP analysis. Plant Breeding:173-179

Oldach K.H., Peck D.M., Cheong J., Williams K.J., Nair R.M. (2008) Identification of a chemically induced point mutation mediating herbicide tolerance in annual medics (Medicago spp.). Ann Bot 101:997-1005

Ott K., Kwagh J., Stockton G.W., Sidorov V., Kakefuda G. (1996) Rational molecular design and genetic engineering of herbicide resistant crops by structure modeling and site-directed mutagenesis of acetohydroxyacid synthase. Journal of Molecular Biology 263:359-368

Ouellet R., Rutledge R.G., Miki B.L. (1992) Members of the acetohydroxyacid synthase multigene family of Brassica napus have divergent patterns of expression. The Plant Journal 2:321-330

Padbury G., Waltman S., Caprio J., Coen G., McGinn S., Mortensen D., Sinclair R. (2002) Agroecosystems and land resources of the northern Great Plains. Agronomy Journal 94:251-261

Pande S., Gan Y., Pathak M., Yadav G. (2007) Integrated crop production and management technology of chickpea. In: Yadav SS, Redden RJ, Chen H (eds.) Chickpea Breeding and Management. CABI, Oxfordshire, UK, pp 277-290

Pang S.S., Duggleby R.G., Guddat L.W. (2002) Crystal structure of yeast acetohydroxyacid synthase: A target for herbicidal inhibitors. Journal of Molecular Biology 317:249–262

Pozniak C.J., Birk I.T., O'Donoughue L.S., Menard C., Hucl P.J., Singh B.K. (2004) Physiological and molecular characterization of mutation-derived imidazolinone resistance in spring wheat. Crop Sci 44:1434-1443

Pozniak C.J., Hucl P.J. (2004) Genetic analysis of imidazolinone resistance in mutation-derived lines of common wheat. Crop Sci 44:23-30

Pundir R.P.S., Mengesha M.H., Reddy K.N. (1990) Leaf types and their genetics in chickpea (*Cicer arietinum* L.). Euphytica 45:197-200

Pundir R.P.S., Rao N.K., van der Maesen L.J.G. (1985) Distribution of qualitative traits in the world germplasm of chickpea (*Cicer arietinum* L.). Euphytica 34:697-703

Rafalski A. (2002) Applications of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology 5:94-100

Rutledge R.G., Quellet T., Hattori J., Miki B.L. (1991) Molecular characterization and genetic origin of the *Brassica napus* acetohydroxyacid synthase multigene family. Molecular and General Genetics 229:31-40

Sala C.A., Bulos M. (2012) Inheritance and molecular characterization of broad range tolerance to herbicides targeting acetohydroxyacid synthase in sunflower. Theor Appl Genet 124:355-364

Sala C.A., Bulos M., Altieri E., Ramos M.L. (2012a) Genetics and breeding of herbicide tolerance in sunflower. Helia 35:57-69

Sala C.A., Bulos M., Altieri E., Weston B. (2012b) Response to imazapyr and dominance relationships of two imidazolinone-tolerant alleles at the Ahasl1 locus of sunflower. Theor Appl Genet 124:385-396

Salimath P.M., Toker C., Sandhu J.S., Kumar J., Suma B., Yadav S.S., Bahl P.N. (2007) Conventional Breeding Methods. In: S.S. Yadav, R.J. Reden, Chen W, Sharma B (eds.) Chickpea Breeding and Management. pp 369-391

Saskatchewan Ministry of Agriculture (2013) Guide to Crop Protection. 3085 Albert Street Regina, Saskatchewan, Canada.

Sathasivan K., Haughn G.W., Murai N. (1990) Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. . Nucleic Acids Research 18:2188

Sathasivan K., Haughn G.W., Murai N. (1991) Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var Columbia. Plant Phys 97:1044-1050

Sethy N.K., Choudhary S., Shokeen B., Bhatia S. (2006) Identification of microsatellite markers from *Cicer reticulatum*: molecular variation and phylogenetic analysis. Theor Appl Genet 112:347-357

Shaner D., Anderson P.C., Stidham M.A. (1984) Imidazolinones. Plant Physiol 76:545-546

Siddique K.H.M., Johansen C., Turner N.C., Jeuffroy M.-H., Hashem A., Sakar D., Gan Y., Alghamdi S.S. (2011) Innovations in agronomy for food legumes. A review. Agronomy for Sustainable Development 32:45-64

Singh B., Shaner D. (1988) Assay of Acetohydroxyacid Synthase. Anal Biochem 171:173-179

Singh K.B. (1997) Chickpea (*Cicer arietinum* L.). Field Crops Research 53:161-170

Smith K., Schloss J.V., Mazur B.J. (1989) Functional expression of plant acetolactate synthase genes in *Escherichia coli*. Proc Natl Acad Sci 86:4179-4183

Soltani A., Robertson M.J., Torabi B., Yousefi-Daz M., Sarparast R. (2006) Modelling seedling emergence in chickpea as influenced by temperature and sowing depth. Agricultural and Forest Meteorology 138:156-167

Statisitcs Canada (2012) National supply and disposition of grains in Canada, 2007/2008 to 2012/2013 — Chickpeas. http://www.statcan.gc.ca/pub/22-002-x/2012006/t073-eng.htm#T073FN1.

Sudupak M.A. (2004) Inter and intra-species Inter Simple Sequence Repeat (ISSR) variations in the genus Cicer. Euphytica 135:229-238

Süzer S., Büyük H. (2010) Residual effects of spraying imidazolinone-family herbicides on Clearfield®* sunflower production from the point of view of crop rotation. Helia 33:25-35

Swanton C.J., Weise S.F. (1991) Integrated Weed Management: The Rationale and Approach. Weed Technol 5:657 -663

Syvanen A.C. (2005) Toward genome-wide SNP genotyping. Nat Genet 37 Suppl:S5-10

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731-2739

Tan S., Evans R.R., Dahmer M.L., Singh B.K., Shaner D.L. (2005) Imidazolinone-tolerant crops: history, current status and future. Pest Manag Sci 61:246-257

Tar'an B., Warkentin T.D., Tullu A., Vandenberg A. (2007) Genetic mapping of ascochyta blight resistance in chickpea (*Cicer arietinum L.*) using a simple sequence repeat linkage map. Genome 50:26-34

Taran B., Holm F., Banniza S. (2013) Response of chickpea cultivars to pre- and post-emergence herbicide applications. Can J of Plant Sci 93:279-286

Taran B., Warkentin T.D., Vandenberg A., Holm F.A. (2010) Variation in chickpea germplasm for tolerance to imazethapyr and imazamox herbicides. Can J of Plant Sci 90:139-142

Toker C., Canci H., Inci N.E., Ceylan F.O. (2012) Improvement in imidazolinone resistance in Cicer species by induced mutation. Plant Breeding 131:535-539

Tranel P.J., Wright T.R. (2002) Resistance of weeds to ALS-inhibiting herbicides what have we learned? Weed Sci 50:700-712

Van der Maesen L.J.G. (ed) (1987) Cicer L. Origin, history and taxonomy of chickpea. The Chickpea. C.A.b. International Cambrian News Ltd, Aberystwyth, UK

Varshney R.K., Song C., Saxena R.K., Azam S., Yu S., Sharpe A.G., Cannon S., Baek J., Rosen B.D., Tar'an B., Millan T., Zhang X., Ramsay L.D., Iwata A., Wang Y., Nelson W., Farmer A.D., Gaur P.M., Soderlund C., Penmetsa R.V., Xu C., Bharti A.K., He W., Winter P., Zhao S., Hane J.K., Carrasquilla-Garcia N., Condie J.A., Upadhyaya H.D., Luo M.C., Thudi M., Gowda C.L., Singh N.P., Lichtenzveig J., Gali K.K., Rubio J., Nadarajan N., Dolezel J., Bansal K.C., Xu X., Edwards D., Zhang G., Kahl G., Gil J., Singh K.B., Datta S.K., Jackson S.A., Wang J., Cook D.R. (2013) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat Biotechnol 31:240-246

Weed Science Society of America (2007) Herbicide Handbook. 9th edn. Weed Science Society of America, Lawrence, KS

White A.D., Graham M.A., Owen M.D.K. (2003) Isolation of acetolactate synthase homologs in common sunflower. Weed Science 51:845-853

Wojciechowski M.F., Lavin M., Sanderson M.J. (2004) A Phylogeny of legumes (Leguminosae) based on anaylsis of the plastid MATK gne resolves many well-suppored subclades within the family. American Journal of Botany 91:1846-1862

Wood J.A., Grusask M.A. (2007) Nutritional Value of Chickpea. In: Yadav SS, Redden RJ, Chen W, Sharma B (eds.) Chickpea Breeding and Managment. CABI, Oxfordshire, pp 101-142

Wright T.R., Penner D. (1998) Cell selection and inheritance of imidazolinone resistance in sugarbeet Theoretical Applied Genetics 96:612-620

Xu Y., Crouch J.H. (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Science 48:391

Yadav G. (2007) Weed Management in Chickpea. In: S.S. Yadav, R.J. Reden, Chen W, Sharma B (eds.) Chickpea Breeding and Management. CABI, Oxfordshire, UK, pp 233-245

Zhou Q., Liu W., Zhang Y., Liu K.K. (2007) Action mechanisms of acetolactate synthase-inhibiting herbicides. Pesticide Biochemistry and Physiology 89:89-96

Appendices

Appendix 1: *Cicer arietinum* L IMI Susceptible Consensus AHAS sequences

Blue = 5' or 3' UTR sequences

Cicer arietinum IMI Susceptible *AHAS1* Nucleotide Sequence

AATGGCCGCCACCACCACCACCACCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATC
TTCCCAAAAAGCAACACCAAACTAACATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCA
TTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGCACCATCCTCCAT
AACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCG
CGTAAAGGCTCCGACATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTT
ACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAACCCGTTCCAAAATAATCCGAAACGT
CCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTT
CCTGGCGTTTGTATTGCCACCTCCGGTCCCGGTGCCACCAACTTAGTTAGCGGTCTCGCCAATG
CTTTGATGGATAGTATTCCAATAATCGCTATCACCGGTCAAGTTCCCCGGAGAATGATCGGAAC
CGATGCTTTTCAAGAAACCCCCATCGTTGAAGTAACGAGATCCATCACAAAGCATAACTACCTC
ATTCTTGAGGTTGATGATATTCCTAGGGTTGTTAGAGAGGCTTTTTTCGTTGCTAATTCTGGTA
GGCCTGGCCCAGTTCTCATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTG
GGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCCCAAGATTCCTATTGAGGCCCAA
TTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTT
GTTTGAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTAC
TTTAATGGGACTAGGTAGTTACCCTGTTGGAGGAGAACATTCCCTTCAAATGTTGGGTATGCAC
GGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGGGGTTAGGT
TTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGA
TATTGATTCGGCTGAAATTGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAG
TTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGATTAAAGATAAACTTGATTTTG
AATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGA
GGATGCGATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATT
GTTAGTACTGGTGTTGGACAGCATCAAATGTGGGCTGCTCAGTTTTATAAGTATAAGAGACCTA
GACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGATGGGCGC
TGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAAT
GTACAAGAGTTAGCTACTATAAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATC
AGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTCGAATAGAGCTCATACTTA
TCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGT
GGGATACCAGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGG
ATACTCCTGGTCCTTATCTTCTAGATGTTATTGTACCTCATCAAGAGCATGTTTTGCCAATGAT
TCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTGATTG
ATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATT
GCTATCCTAGTTGCGGGATTTGACACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTG
TAATTTTGGTGGCATGTTTCTTTGTAGAATGCCGCACCTCTTGTTGTTGTATTGTTTTTTTTTT
CTTTTCTGTA

***Cicer arietinum* IMI Susceptible AHAS1 Amino Acid Sequence**

MAATTTTTPSRSAFSSSSHPIFPKSNTKLTFSLSPIFNKPKLISSRPFKISSSLSKSPTA
PSSITTTTTTTTTSTFISRFSPTEPRKGSDILVEALEREGVTNVFAYPGGASMEIHQALT
RSKIIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSGLANALMDSIPIIA
ITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILEVDDIPRVVREAFFVANSGRPGPVL
IDVPKDVQQQLAVPNWAQPIKLTGYLSRLPKIPIEAQLEQVVRLLLESKKPVLYVGGGCL
NSSEELKRFVEITGIPVASTLMGLGSYPVGGEHSLQMLGMHGTVYANYAVDKSDLLLAFG
VRFDDRVTGKLETFASRANIVHIDIDSAEIGKNKLPQVSVCADMKFALQGLNRILESKGI
KDKLDFESWREELNVVKIKFPLGFKTFEDAISPQYAIQVLDELTNGDAIVSTGVGQHQMW
AAQFYKYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDAVVVDIDGDGSFMMNVQELATI
KVEKLPVKILLLNNQHLGMVVQWEDRFYKSNRAHTYLGDPSRENEIFPNMLGFADACGIP
AARVTKKEELRDAIQKMLDTPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITDGDGRRSY-
LIGLN-IRYSFTVVLYNIYSYYCYPSCGI-HSL-AKHVSLVFCCNFGGMFLCRMPHLLLL
YCFFFLFC

***Cicer arietinum* IMI Susceptible *AHAS2* Nucleotide Sequence**

ATGGCGACCAACGCTGCTAGAGCCGCATTCACCACCGTGCCTTTATCTTCACCACATTCTCCAA
ACCCAAACAGCATTTTCCGATTCACCGTTCCGTTTTCGTCACACTTAAACACTCCCTCCAAACT
CCAACTCCAACTCCGTTCTCTCCGAATCTCCGCTTCCCTCTCAAACAACCCTACACACCAACTC
ACTCCCCTCCCCTCAACCGAACAATTCATTTCCCGATTTGCCCTTGACGAACCTCGAAAAGGCG
CCGACATCCTCGTCGAAGCTCTCGAACGTCAAGGCGTCACCAATGTCTTCGCCTATCCCGGCGG
CGCGTCAATGGAAATTCACCAAGCACTCACGCGCTCCACCACCATCCGGAACGTCCTTCCCCGC
CACGAACAAGGTGGAATTTTCGCCGCTGAGGGTTACGCTCGTTCCTCCGGCCTCCCTGGTGTCT
GCATCGCCACCTCCGGTCCCGGCGCCACTAACCTCGTCAGCGGTCTCGCCGACGCTATGCTCGA
TAGCGTTCCTCTCGTCGCTATCACCGGTCAAGTCCCCCGGAGAATGATCGGTACCGATGCATTT
CAAGAAACTCCGATTGTTGAGGTAACTAGGTCAATTACAAAGCACAATTATCTTGTTCTGGATA
TTGATGATATACCTAGGATAGTGAATGAAGCTTTTTTCTTAGCTACTTCTGGAAGACCTGGACC
TGTGTTAATTGATATACCTAAAGATATTCAACAACAACTTTCGGTTCCAAATTGGGAACAACCT
ATGAAGTTATCAGGGTATATGGCTAGGTTACCAAAGTCACCTTCTGAGTCACATTTAGAACAGA
TTGTGAGGTTGATAATGGAATCTAAGAAACCTGTTTTATATGTTGGTGGTGGTAGTTTGAATTC
TAGTGAGGAATTGAGGCGTTTCGTGGAGTTAACTGGTGTTCCTGTTGCTAGTACTTTGATGGGT
TTGGGTTCATACCCTACTTCTGATGAGAATTCACTTCAAATGCTTGGTATGCATGGAACTGTTT
ATGCTAATTATGCTGTTGATAAGAGTGATCTTTTGCTTGCGTTTGGGGTTCGGTTTGATGATCG
TGTTACCGGGAAGCTTGAGGCTTTTGCTAGTCGGGCGAAGATTGTTCATATCGATATCGATTCT
GCTGAGATTGGGAAAAACAAGCAGCCTCACGTGTCAGTTTGTGGGGATTTAAAGTTGGCTTTGA
GGGGGATTAATAGGATTTTGGAGAGTAAAGGGATAGAGAGTAAGCTTGATTTTGGAGCTTGGAG
AGAAGAGTTGAATGAACAGAAACGTAGATTTCCGTTGAGTTTTAAGACGTTTGGTGAAGCTATT
CCTCCACAGTATGCTATACAGGTTCTTGATGAGCTTACCAATGGAGATGCTATCATAAGTACTG
GGGTTGGACAGCATCAGATGTGGTCTGCTCAATTTTATAGTTATAAGAGACCTAGGCAGTGGTT
GACTTCAGGTGGTCTTGGTGCTATGGGATTTGGATTACCTGCTGCCATGGGAGCTGCTGTTGCT
AATCCTGACTCTGTTGTGGTTGACATCGATGGTGATGGAAGTTTTATGATGAATGTTCAGGAGC

TGGCTACAATAAAGGTAGAGAATCTCCCTGTAAAGATATTGTTGCTGAACAATCAACACTTGGG
TATGGTTGTTCAGTGGGAGGATCGGTTCTATAAAGCCAATAGAGCTCACACCTATCTGGGAGAC
CCGGCTAACGAGAAAGAAATATTCCCTAATATGTTGAAATTTGCAGATGCTTGTGGTATACCGG
CAGCTCGTGTGACAAAGAAGGCAGACCTTAGAGCCGCAATTCAGAAAATGCTGGATACCCCTGG
CCCCTACCTTCTTGATGTCATTGTACCCCATCAAGAGCATGTGTTGCCAATGATTCCCAGTAAT
GGAGCCTTCGAGGATGTGATAACTGAAGGTGATGGCAGGAGAAGTTACTGATTTCCCGAGCCAG
GTTTATCTTGATGCCGATTATGTATAATAGGAAGCGATAATGTTATAGTTTTTGTTTTAGTGTT
GGCACTAAGACAGGCCCATTTGTTTCTATACAATTTTCGTGTCCCTTATAAAATTACCTACTGC
TCTAATAAGTTATAACTGCGGTTTTAAATTGTATTGCTGATGCCGAAT

Cicer arietinum IMI Susceptible AHAS2 Amino Acid Sequence

MATNAARAAFTTVPLSSPHSPNPNSIFRFTVPFSSHLNTPSKLQLQLRSLRISASLSNNP
THQLTPLPSTEQFISRFALDEPRKGADILVEALERQGVTNVFAYPGGASMEIHQALTRST
TIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSGLADAMLDSVPLVAITG
QVPRRMIGTDAFQETPIVEVTRSITKHNYLVLDIDDIPRIVNEAFFLATSGRPGPVLIDI
PKDIQQQLSVPNWEQPMKLSGYMARLPKSPSESHLEQIVRLIMESKKPVLYVGGGSLNSS
EELRRFVELTGVPVASTLMGLGSYPTSDENSLQMLGMHGTVYANYAVDKSDLLLAFGVRF
DDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVSVCGDLKLALRGINRILESKGIESK
LDFGAWREELNEQKRRFPLSFKTFGEAIPPQYAIQVLDELTNGDAIISTGVGQHQMWSAQ
FYSYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDSVVVDIDGDGSFMMNVQELATIKVE
NLPVKILLLNNQHLGMVVQWEDRFYKANRAHTYLGDPANEKEIFPNMLKFADACGIPAAR
VTKKADLRAAIQKMLDTPGPYLLDVIVPHQEHVLPMIPSNGAFEDVITEGDGRRSY-FPE
PGLS-CRLCIIGSDNVIVFVLVLALRQAHLFLYNFRVPYKITYCSNKL-LRF-IVLLMPN

Appendix 2: *Cicer arietinum* L. IMI Tolerant Consensus AHAS sequences

Cicer arietinum IMI Tolerant *AHAS1* Nucleotide Consensus Sequence

CCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACT
AACATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATC
TCATCCTCCCTCTCAAAATCCCCCACCGCACCATCCTCCATAACAACAACAACAACAACCACCA
CCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGACATTCTTGT
CGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAG
ATCCACCAAGCCCTAACCCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCG
GTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCCTGGCGTTTGTATTGCCACCTC
CGGTCCCGGTGCCACCAACTTAGTTAGCGGTCTCGCCAATGCTTTGATGGATAGTATTCCAATA
ATCGCTATCACCGGTCAAGTTCCCCGGAGAATGATCGGAACCGATGTTTTTCAAGAAACCCCCA
TCGTTGAAGTAACGAGATCCATCACAAAGCATAACTACCTCATTCTTGAGGTTGATGATATTCC
TAGGGTTGTTAGAGAGGCTTTTTTCGTTGCTAATTCTGGTAGGCCTGGCCCAGTTCTCATTGAC
GTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTG
GATATCTCTCCAGGCTTCCCAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATT
ATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTTGAATTCAAGTGAGGAGTTA
AAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACC

CTGTTGGAGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGC
TGTTGATAAAAGTGATTTATTGCTTGCTTTTGGGGTTAGGTTTGATGATCGTGTAACTGGGAAA
TTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAATTGGGA
AGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAG
GATTTTGGAGAGTAAAGGGATTAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAAT
GTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGCGATTTCACCTCAGTATG
CCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCA
TCAAATGTGGGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGA
CTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGATGGGCGCTGCTGTTGCTAACCCTGATGCTG
TTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTATAAA
AGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAG
TGGGAGGATAGATTCTACAAGTCGAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGA
ATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACCAGCAGCTCGTGTGAC
GAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTA
GATGTTATTGTACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGG
ATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTGATTGATTGGGCTAAACTAGATACGGTA
TTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTGA
CACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTGTAATTTTGGTGGCATGTTTCTTT
GTAGAATGCCGCACCTCTTGTTGTTGTATTGTTTT

Cicer arietinum IMI Tolerant AHAS1 Amino Acid Sequence

PSRSAFSSSSHPIFPKSNTKLTFSLSPIFNKPKLISSRPFKISSSLSKSPTAPSSITTTT
TTTTTSTFISRFSPTEPRKGSDILVEALEREGVTNVFAYPGGASMEIHQALTRSKIIRNV
LPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSGLANALMDSIPIIAITGQVPRR
MIGTDVFQETPIVEVTRSITKHNYLILEVDDIPRVVREAFFVANSGRPGPVLIDVPKDVQ
QQLAVPNWAQPIKLTGYLSRLPKIPIEAQLEQVVRLLLESKKPVLYVGGGCLNSSEELKR
FVEITGIPVASTLMGLGSYPVGGEHSLQMLGMHGTVYANYAVDKSDLLLAFGVRFDDRVT
GKLETFASRANIVHIDIDSAEIGKNKLPQVSVCADMKFALQGLNRILESKGIKDKLDFES
WREELNVVKIKFPLGFKTFEDAISPQYAIQVLDELTNGDAIVSTGVGQHQMWAAQFYKYK
RPRQWLTSGGLGAMGFGLPAAMGAAVANPDAVVVDIDGDGSFMMNVQELATIKVEKLPVK
ILLLNNQHLGMVVQWEDRFYKSNRAHTYLGDPSRENEIFPNMLGFADACGIPAARVTKKE
ELRDAIQKMLDTPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITDGDGRRSY-LIGLN-IR
YSFTVVLYNIYSYYCYPSCGI-HSL-AKHVSLVFCCNFGGMFLCRMPHLLLLYCF

***Cicer arietinum* IMI Tolerant *AHAS2* Consensus Nucleotide Sequence**

AAAACAATAGAGATTTTAAAGGCCTGCATTTTGGAACTGTTTCATTCAACAGARAAATGGCGAC
CAACGCTGCTAGAGCCGCATTCACCACCGTGCCTTTATCTTCACCACATTCTCCAAACCCAAAC
AGCATTTTCCGATTCACCGTTCCGTTTTCGTCACACTTAAACACTCCCTCCAAACTCCAACTCC
AACTCCGTTCTCTCCGAATCTCCGCTTCCCTCTCAAACAACCCTACACACCAACTCACTCCCCT
CCCCTCAACCGAACAATTCATTTCCCGATTTGCCCTTGACGAACCTCGAAAAGGCGCCGACATC
CTCGTCGAAGCTCTCGAACGTCAAGGCGTCACCAATGTCTTCGCCTATCCCGGCGGCGCGTCAA
TGGAAATTCACCAAGCACTCACGCGCTCCACCACCATCCGGAACGTCCTTCCCCGCCACGAACA
AGGTGGAATTTTCGCCGCTGAGGGTTACGCTCGTTCCTCCGGCCTCCCTGGTGTCTGCATCGCC
ACCTCCGGTCCCGGCGCCACTAACCTCGTCAGCGGTCTCGCCGACGCTATGCTCGATAGCGTTC
CTCTCGTCGCTATCACCGGTCAAGTCCCCCGGAGAATGATCGGTACCGATGCATTTCAAGAAAC
TCCGATTGTTGAGGTAACTAGGTCAATTACAAAGCACAATTATCTTGTTCTGGATATTGATGAT
ATACCTAGGATAGTGAATGAAGCTTTTTTCTTAGCTACTTCTGGAAGACCTGGACCTGTGTTAA
TTGATATACCTAAAGATATTCAACAACAACTTTCGGTTCCAAATTGGGAACAACCTATGAAGTT
ATCAGGGTATATGGCTAGGTTACCAAAGTCACCTTCTGAGTCACATTTAGAACAGATTGTGAGG
TTGATAATGGAATCTAAGAAACCTGTTTTATATGTTGGTGGTGGTAGTTTGAATTCTAGTGAGG
AATTGAGGCGTTTCGTGGAGTTAACTGGTGTTCCTGTTGCTAGTACTTTGATGGGTTTGGGTTC
ATACCCTACTTCTGATGAGAATTCACTTCAAATGCTTGGTATGCATGGAACTGTTTATGCTAAT
TATGCTGTTGATAAGAGTGATCTTTTGCTTGCGTTTGGGGTTCGGTTTGATGATCGTGTTACCG
GGAAGCTTGAGGCTTTTGCTAGTCGGGCGAAGATTGTTCATATCGATATCGATTCTGCTGAGAT
TGGGAAAAACAAGCAGCCTCACGTGTCAGTTTGTGGGGATTTAAAGTTGGCTTTGAGGGGGATT
AATAGGATTTTGGAGAGTAAAGGGATAGAGAGTAAGCTTGATTTTGGAGCTTGGAGAGAAGAGT
TGAATGAACAGAAACGTAGATTTCCGTTGAGTTTTAAGACGTTTGGTGAAGCTATTCCTCCACA
GTATGCTATACAGGTTCTTGATGAGCTTACCAATGGAGATGCTATCATAAGTACTGGGGTTGGA
CAGCATCAGATGTGGTCTGCTCAATTTTATAGTTATAAGAGACCTAGGCAGTGGTTGACTTCAG
GTGGTCTTGGTGCTATGGGATTTGGATTACCTGCTGCCATGGGAGCTGCTGTTGCTAATCCTGA
CTCTGTTGTGGTTGACATCGATGGTGATGGAAGTTTTATGATGAATGTTCAGGAGCTGGCTACA
ATAAAGGTAGAGAATCTCCCTGTAAAGATATTGTTGCTGAACAATCAACACTTGGGTATGGTTG
TTCAGTGGGAGGATCGGTTCTATAAAGCCAATAGAGCTCACACCTATCTGGGAGACCCGGCTAA
CGAGAAAGAAATATTCCCTAATATGTTGAAATTTGCAGATGCTTGTGGTATACCGGCAGCTCGT
GTGACAAAGAAGGCAGACCTTAGAGCCGCAATTCAGAAAATGCTGGATACCCCTGGCCCCTACC
TTCTTGATGTCATTGTACCCCATCAAGAGCATGTGTTGCCAATGATTCCCAGTAATGGAGCCTT
CGAGGATGTGATAACTGAAGGTGATGGCAGGAGAAGTTACTGATTTCCCGAGCCAGGTTTATCT
TGATGCCGATTATGTATAATAGGAAGCGATAATGTTATAGTTTTTGTTTTAGTGTTGGCACTAA
GACAGGCCCATTTGTTTCTATACAATTTTCGTGTCCCTTATAAAATTACCTACTGCTCTAATAA
GTTATAACTGCGGTTTTAAAT

***Cicer arietinum* IMI Resistant AHAS2 Amino Acid Consensus Sequence**

NNRDFKGLHFGTVSFNRXMATNAARAAFTTVPLSSPHSPNPNSIFRFTVPFSSHLNTPSK
LQLQLRSLRISASLSNNPTHQLTPLPSTEQFISRFALDEPRKGADILVEALERQGVTNVF
AYPGGASMEIHQALTRSTTIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLV
SGLADAMLDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVLDIDDIPRIVN
EAFFLATSGRPGPVLIDIPKDIQQQLSVPNWEQPMKLSGYMARLPKSPSESHLEQIVRLI
MESKKPVLYVGGGSLNSSEELRRFVELTGVPVASTLMGLGSYPTSDENSLQMLGMHGTVY
ANYAVDKSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVSVCGDLK
LALRGINRILESKGIESKLDFGAWREELNEQKRRFPLSFKTFGEAIPPQYAIQVLDELTN
GDAIISTGVGQHQMWSAQFYSYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDSVVVDID
GDGSFMMNVQELATIKVENLPVKILLLNNQHLGMVVQWEDRFYKANRAHTYLGDPANEKE
IFPNMLKFADACGIPAARVTKKADLRAAIQKMLDTPGPYLLDVIVPHQEHVLPMIPSNGA
FEDVITEGDGRRSY-FPEPGLS-CRLCIIGSDNVIVFVLVLALRQAHLFLYNFRVPYKIT
YCSNKL-LRF-

Appendix 3: *Lens culinaris* Consensus AHAS sequences

***Lens Culinaris* CDC Redberry (IMI Susceptible) *AHAS1* Consensus Nucleotide Sequence**

GCATCGCCACTTCCGGTCCTGGTGCCACCAATTTAGTCAGCGGCCTCGCCGATGCTTTGATGGA
CAGTGTCCCTCTTGTCGCCATTACCGGTCAAGTTCCCCGGAGAATGATTGGAACTGATGCTTTT
CAAGAAACCCCAATCGTTGAAGTAACGAGATCTATTACAAAGCATAACTACCTCATTCTAGATG
TTGATGATATTCCTAGGGTTGTGAAAGAGGCTTTTTTCCTTGCAACTTCAGGTAGGCCCGGCCC
GGTTCTCATCGACGTACCCAAAGACATTCAACAACAGTTAGCAGTTCCGAATTGGGCCGAGCCC
ATTAAGCTCACCGGATATGTTTCCAGGTTACCAAAGATTCCTGATGAGTCCCAATTTGAACAGG
TTGTGAGGTTATTATTAGAATCTAAGAAACCTGTTTTGTATGTTGGAGGGGGTTGCTTGAATTC
CAGCGAGGAATTGAACCGGTTTGTTGAACTAACCGGCATTCCTGTTGCTAGTACTTTAATGGGA
CTTGGTAGTTACCCTATTGGAGGTGAACATTCCCTTCATATGTTGGGTATGCATGGTACTGTTT
ATGCTAATTATGCTGTTGATAGTAGTGATTTGTTACTTGCTTTCGGGGTTAGGTTTGATGATCG
AGTAACCGGAAAGTTAGAAGCTTTTGCGAGTAGGGCTAAGATTGTTCATATAGATATTGATTCA
GCTGAGATTGGGAAGAATAAGATTCCACATATGTCGATTTGTGCTGATATGAAAGTGGCATTGG
AAGGTCTTAATAGGGTTTTGGAGAGTAAAGGAGTCAAAGGTAAACTTGATTTTGAAGCATGGAG
GCAGGAGTTGAATGTTCAGAAATTGAAATTTCCTCTTGGGTTTAAGACGTTTGAGAATGCGATT
TCTCCTCAGTATGCTATTCAGGTGCTTGATGAATTGACTAATGGAGATGCTATTATTAGTACTG
GTGTTGGACAACATCAAATGTGGGCTGCTCAGTTTTATGAGTATAAGAGACCTAGGCAGTGGTT
AACTTCTGGTGGACTCGGTGCTATGGGTTTTGGATTACCGGCTGCTATTGGTGCTGCTGTTGCT
AACCCCAATGCGGTTGTTGTTGATATTGATGGCGATGGTAGCTTTATAATGAATGTTCAAGAGT
TGGCTACTATAAGAGTGGAGAATCTTCCTATTAAGATATTGTTGTTGAATAATCAACATTTGGG
TATGGTTGTTCAATGGGAGGATAGATTCTATAAGTCAAATAGAGGTCATACTTATCTTGGTGAC
CCTTCTAGGGAGGAAGAGATTTTCCCTAATATGCTTGGATTTGCTGATGCTTGTGGAATACCGG
CGGCTCGTGTGACGAAGAAGGAAGAGCTTCGAGAAGCTATTCAGAAAATGTTGGATACTCCTGG
CCCTTATCTTCTTGATGTCATTACACCTCATCAAGAACATGTATTGCCAATGATTCCAAGTAAT

GGATCTTTCAAGGATGTGATCACTGAGGGTGATGGCAGAACGAGCTACTGATTTCTTGGTCCAA
AATAAGATATGATATTCCTTCATGTCTGTTTTGTACAATAGATATAACTAATGCTATCATAGTT
ATAGGATTTAATGGCTTAAGTTGATCCAATTAGCTTACTCCACCTAATGGGATAAGGCTTAATT
GTTGTGGTTGTTGTCGTCGTCGTCGTCATAGTTACAGGATTTTACACGGGGTAAGTTAAGCAGT
GTATTTTGATGTAATTTTTGGTGGCATGTGTCTTTGTAGAGGGAACATTATGGCGAAAGTTGAT
CCATGTAGTCAATTCTATTTAGTTGTATAAGACTTGGTTGTTTTGTTGATCTGTCTTTTAGAGT
GTCAT

***Lens culinaris* CDC Redberry (IMI Susceptible) AHAS1 Consensus Amino Acid Sequence**

IATSGPGATNLVSGLADALMDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYL
ILDVDDIPRVVKEAFFLATSGRPGPVLIDVPKDIQQQLAVPNWAEPIKLTGYVSRLPKIP
DESQFEQVVRLLLESKKPVLYVGGGCLNSSEELNRFVELTGIPVASTLMGLGSYPIGGEH
SLHMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKN
KIPHMSICADMKVALEGLNRVLESKGVKGKLDFEAWRQELNVQKLKFPLGFKTFENAISP
QYAIQVLDELTNGDAIISTGVGQHQMWAAQFYEYKRPRQWLTSGGLGAMGFGLPAAIGAA
VANPNAVVVDIDGDGSFIMNVQELATIRVENLPIKILLLNNQHLGMVVQWEDRFYKSNRG
HTYLGDPSREEEIFPNMLGFADACGIPAARVTKKEELREAIQKMLDTPGPYLLDVITPHQ
EHVLPMIPSNGSFKDVITEGDGRTSY-FLGPK-DMIFLHVCFVQ-I-LMLS-L-DLMA-V
DPISLLHLMG-GLIVVVVVVVVVIVTGFYTG-VKQCILM-FLVACVFVEGTLWRKLIHVV
NSI-LYKTWLFC-SVF-SV

***Lens culinaris* CDC Impact (IMI Resistant) *AHAS1* Consensus Nucleotide Sequence**

CTTTTTATATTGTGAAACAAATATCTAGAAAGTAAATTAACACACGTGGATTATCACGCGGCGA
GATCCACACCACAAAATGAAGCCCCATCGACAGCAGTTGACTATTTTACCCTCAACACACCGAT
CCATTTGACAAAAAGACAAGGGGCAAGAAAGTAAAGAAAGAAGCAAAACAACCTCAGTCACATA
GTATGATAGTACTATACTATAAACTAGACAAGACACCTGAATATTCATATTATTACACTTCAAA
CTCAAACTCTACTTGCATTCAACCATGGCCGCCGCCGCCGCCACCACCACCACCACCACTTCCA
GATCTCCGTTCACTTCATCTTCTTCTTCGTATTCCACTTTCCTTAAACGAAACTCAACACTCAC
ACTCCCTTTCTCTCCCATTTACAACAAACCACAGTCCATTCACAATCGACCACTCACTGTCTCA
TCATCCCTCTCCAACTACCCCGTCGCACCAGCTTCCACCACCGCCACCACCCCCGATGATCAAT
ACATCTCTCGTTTCTCCTCCACCGAGCCAAGAAAAGGCGCTGACATTCTCGTCGAAGCTCTCGA
GCGTCAAGGCGTCACCAACGTATTCGCTTACCCCGGCGGAGCTTCCATGGAGATCCACCAAGCT
CTTACACGCTCCAAAACAATCCGAAACATACTTCCCCGTCACGAACAAGGTGGAGTCTTCGCCG
CCGAAGGCTACGCGCGCTCCTCCGGTCTCCCCGGTGTTTGCATCGCCACTTCCGGTCCTGGTGC
CACCAATTTAGTCAGCGGCCTCGCCGATGCTTTGATGGACAGTGTCCCTCTTGTCGCCATTACC
GGTCAAGTTCCCCGGAGAATGATTGGAACTGATGTTTTTCAAGAAACCCCAATCGTTGAAGTAA
CGAGATCTATTACAAAGCATAACTACCTCATTCTAGATGTTGATGATATTCCTAGGGTTGTGAA
AGAGGCTTTTTTCCTTGCAACTTCAGGTAGGCCCGGCCCGGTTCTCATCGACGTACCCAAAGAC
ATTCAACAACAGTTAGCAGTTCCGAATTGGGCCGAGCCCATTAAGCTCACCGGATATGTTTCCA
GGTTACCAAAGATTCCTGATGAGTCCCAATTTGAACAGGTTGTGAGGTTATTATTAGAATCTAA
GAAACCTGTTTTGTATGTTGGAGGGGGTTGCTTGAATTCCAGCGAGGAATTGAACCGGTTTGTT

GAACTAACCGGCATTCCTGTTGCTAGTACTTTAATGGGACTTGGTAGTTACCCTATTGGAGGTG
AACATTCCCTTCATATGTTGGGTATGCATGGTACTGTTTATGCTAATTATGCTGTTGATAGTAG
TGATTTGTTACTTGCTTTCGGGGTTAGGTTTGATGATCGAGTAACCGGAAAGTTAGAAGCTTTT
GCGAGTAGGGCTAAGATTGTTCATATAGATATTGATTCAGCTGAGATTGGGAAGAATAAGATTC
CACATATGTCGATTTGTGCTGATATGAAAGTGGCATTGGAAGGTCTTAATAGGGTTTTGGAGAG
TAAAGGAGTCAAAGGTAAACTTGATTTTGAAGCATGGAGGCAGGAGTTGAATGTTCAGAAATTG
AAATTTCCTCTTGGGTTTAAGACGTTTGAGAATGCGATTTCTCCTCAGTATGCTATTCAGGTGC
TTGATGAATTGACTAATGGAGATGCTATTATTAGTACTGGTGTTGGACAACATCAAATGTGGGC
TGCTCAGTTTTATGAGTATAAGAGACCTAGGCAGTGGTTAACTTCTGGTGGACTCGGTGCTATG
GGTTTTGGATTACCGGCTGCTATTGGTGCTGCTGTTGCTAACCCCAATGCGGTTGTTGTTGATA
TTGATGGCGATGGTAGCTTTATAATGAATGTTCAAGAGTTGGCTACTATAAGAGTGGAGAATCT
TCCTATTAAGATATTGTTGTTGAATAATCAACATTTGGGTATGGTTGTTCAATGGGAGGATAGA
TTCTATAAGTCAAATAGAGGTCATACTTATCTTGGTGACCCTTCTAGGGAGGAAGAGATTTTCC
CTAATATGCTTGGATTTGCTGATGCTTGTGGAATACCGGCGGCTCGTGTGACGAAGAAGGAAGA
GCTTCGAGAAGCTATTCAGAAAATGTTGGATACTCCTGGCCCTTATCTTCTTGATGTCATTACA
CCTCATCAAGAACATGTATTGCCAATGATTCCAAGTAATGGATCTTTCAAGGATGTGATCACTG
AGGGTGATGGCAGAACGAGCTACTGATTTCTTGGTCCAAAATAAGATATGATATTCCTTCATGT
CTGTTTTGTACAATAGATATAACTAATGCTATCATAGTTATAGGATTTAATGGCTTAAGTTGAT
CCAATTAGCTTACTCCACCTAATGGGATAAGGCTTAATTGTTGTGGTTGTTGTCGTCGTCGTCG
TCATAGTTACAGGATTTTACACGGGGTAAGTTAAGCAGTGTATTTTGATGTAATTTTTGGTGGC
ATGTGTCTTTGTAGAGGGAACATTATGGCGAAAGTTGATCCATGTAGTCAATTCTATTTAGTTG
TATAAGACTTGGTTGTTTTGTTGATCTGTCTTTTAGAGTGTC

Lens culinaris CDC Impact (IMI Resistant) AHAS1 Consensus Amino Acid Sequence

FLYCETNI-KVN-HTWIITRRDPHHKMKPHRQQLTILPSTHRSI-QKDKGQESKERSKTT
SVT-YDSTIL-TRQDT-IFILLHFKLKLYLHSTMAAAAATTTTTTSRSPFTSSSSSYSTF
LKRNSTLTLPFSPIYNKPQSIHNRPLTVSSSLSNYPVAPASTTATTPDDQYISRFSSTEP
RKGADILVEALERQGVTNVFAYPGGASMEIHQALTRSKTIRNILPRHEQGGVFAAEGYAR
SSGLPGVCIATSGPGATNLVSGLADALMDSVPLVAITGQVPRRMIGTDVFQETPIVEVTR
SITKHNYLILDVDDIPRVVKEAFFLATSGRPGPVLIDVPKDIQQQLAVPNWAEPIKLTGY
VSRLPKIPDESQFEQVVRLLLESKKPVLYVGGGCLNSSEELNRFVELTGIPVASTLMGLG
SYPIGGEHSLHMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDI
DSAEIGKNKIPHMSICADMKVALEGLNRVLESKGVKGKLDFEAWRQELNVQKLKFPLGFK
TFENAISPQYAIQVLDELTNGDAIISTGVGQHQMWAAQFYEYKRPRQWLTSGGLGAMGFG
LPAAIGAAVANPNAVVVDIDGDGSFIMNVQELATIRVENLPIKILLLNNQHLGMVVQWED
RFYKSNRGHTYLGDPSREEEIFPNMLGFADACGIPAARVTKKEELREAIQKMLDTPGPYL
LDVITPHQEHVLPMIPSNGSFKDVITEGDGRTSY-FLGPK-DMIFLHVCFVQ-I-LMLS-
L-DLMA-VDPISLLHLMG-GLIVVVVVVVVVIVTGFYTG-VKQCILM-FLVACVFVEGTL
WRKLIHVVNSI-LYKTWLFC-SVF-S

Appendix 4: Chickpea (*Cicer arietinum* L.) *AHAS1* nucleotide sequence alignment of IMI-S and IMI-R genotypes

Highlighed areas indicate mutations site conferring to Group 2 Imidazolinone resistance,
IMI-S genotypes→ MylesCDC Corinne, CDC Frontier and CDC Luna
IMI-R genotypes →ICCX860047-9, CDC Cory, and CDC Alma

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CDC Corinne     -ATGGCCGCCACCACCACCACCACCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
CDC Luna        ---GGCCGCCACCACCACCACCACCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
CDC Cory        ----------------------CCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
CDC Frontier    AATGGCCGCCACCACCACCACCACCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
ICCX860047-9    ----------------------CCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
Myles           ---GGCCGCCACCACCACCACCACCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
CDC Alma        ----------------------CCCCTTCCAGATCCGCTTTCTCTTCTTCTTCACATCCTATCTTCCCAAAAAGCAACACCAAACTAAC
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CDC_Corinne     ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
CDC_Luna        ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
CDC_Cory        ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
CDC_Frontier    ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
ICCX860047-9    ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
Myles           ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
CDC_Alma        ATTCTCTCTCTCTCCCATTTTCAACAAACCAAAACTCATTAGTTCTCGTCCCTTCAAAATCTCATCCTCCCTCTCAAAATCCCCCACCGC
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CDC_Corinne     ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
CDC_Luna        ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
CDC_Cory        ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
CDC_Frontier    ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
ICCX860047-9    ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
Myles           ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
CDC_Alma        ACCATCCTCCATAACAACAACAACAACAACCACCACCACCTCCACTTTCATATCTCGCTTCTCACCCACCGAACCGCGTAAAGGCTCCGA
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CDC_Corinne     CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
CDC_Luna        CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
CDC_Cory        CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
CDC_Frontier    CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
ICCX860047-9    CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
Myles           CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
CDC_Alma        CATTCTTGTCGAAGCTTTAGAACGCGAAGGCGTAACCAACGTCTTCGCTTACCCCGGTGGAGCTTCCATGGAGATCCACCAAGCCCTAAC
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CDC_Corinne CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
CDC_Luna CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
CDC_Cory CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
CDC_Frontier CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
ICCX860047-9 CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
Myles CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
CDC_Alma CCGTTCCAAAATAATCCGAAACGTCCTTCCCCGTCACGAACAAGGCGGTATATTCGCCGCCGAAGGCTACGCGCGTTCCTCCGGTCTTCC
TGGCGTTTGTATTGCCACCTCCGGTCCCGGTGCCACCAACTTAGTTAGCGGTCTCGCCAATGCTTTGATGGATAGTATTCCAATAATCGC
TATCACCGGTCAAGTTCCCCGGAGAATGATCGGAACCGATGCTTTTCAAGAAACCCCCATCGTTGAAGTAACGAGATCCATCACAAAGCA
TATCACCGGTCAAGTTCCCCGGAGAATGATCGGAACCGATGTTTTTCAAGAAACCCCCATCGTTGAAGTAACGAGATCCATCACAAAGCA
TAACTACCTCATTCTTGAGGTTGATGATATTCCTAGGGTTGTTAGAGAGGCTTTTTTCGTTGCTAATTCTGGTAGGCCTGGCCCAGTTCT

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CDC_Corinne     CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
CDC_Luna        CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
CDC_Cory        CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
CDC_Frontier    CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
ICCX860047-9    CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
Myles           CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
CDC_Alma        CATTGACGTACCCAAAGACGTTCAACAACAACTCGCTGTTCCGAATTGGGCCCAGCCCATTAAACTAACTGGATATCTCTCCAGGCTTCC
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CDC_Corinne     CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
CDC_Luna        CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
CDC_Cory        CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
CDC_Frontier    CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
ICCX860047-9    CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
Myles           CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
CDC_Alma        CAAGATTCCTATTGAGGCCCAATTAGAACAAGTTGTTCGGTTATTATTAGAATCTAAAAAACCTGTTTTATATGTTGGAGGTGGTTGTTT
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CDC_Corinne     GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
CDC_Luna        GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
CDC_Cory        GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
CDC_Frontier    GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
ICCX860047-9    GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
Myles           GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGGTATTCCTGTTGCTAGTACTTTAATGGGACTAGGTAGTTACCCTGTTGG
CDC_Alma        GAATTCAAGTGAGGAGTTAAAACGGTTTGTTGAAATTACCGG----------------------------------------------
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CDC_Corinne     AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
CDC_Luna        AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
CDC_Cory        AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
CDC_Frontier    AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
ICCX860047-9    AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
Myles           AGGAGAACATTCCCTTCAAATGTTGGGTATGCACGGTACTGTTTATGCTAATTATGCTGTTGATAAAAGTGATTTATTGCTTGCTTTTGG
CDC_Alma        --------------------------------------------------------------------------------------
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CDC_Corinne     GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
CDC_Luna        GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
CDC_Cory        GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
CDC_Frontier    GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
ICCX860047-9    GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
Myles           GGTTAGGTTTGATGATCGTGTAACTGGGAAATTAGAAACTTTTGCTAGTAGGGCTAATATTGTTCATATAGATATTGATTCGGCTGAAAT
CDC_Alma        ----------------------------------------------------------------------------------AT
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CDC_Corinne     TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
CDC_Luna        TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
CDC_Cory        TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
CDC_Frontier    TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
ICCX860047-9    TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
Myles           TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
CDC_Alma        TGGGAAGAATAAGCTTCCACAAGTGTCTGTTTGTGCTGACATGAAGTTTGCGTTACAAGGTCTTAATAGGATTTTGGAGAGTAAAGGGAT
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CDC_Corinne     TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
CDC_Luna        TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
CDC_Cory        TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
CDC_Frontier    TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
ICCX860047-9    TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
Myles           TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
CDC_Alma        TAAAGATAAACTTGATTTTGAATCATGGAGAGAGGAATTGAATGTTGTGAAAATTAAATTTCCTCTTGGGTTTAAGACGTTTGAGGATGC
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CDC_Corinne     GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
CDC_Luna        GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
CDC_Cory        GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
CDC_Frontier    GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
ICCX860047-9    GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
Myles           GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
CDC_Alma        GATTTCACCTCAGTATGCCATCCAGGTGCTCGATGAATTGACAAATGGTGATGCTATTGTTAGTACTGGTGTTGGACAGCATCAAATGTG
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CDC_Corinne     GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
CDC_Luna        GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
CDC_Cory        GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
CDC_Frontier    GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
ICCX860047-9    GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
Myles           GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
CDC_Alma        GGCTGCTCAGTTTTATAAGTATAAGAGACCTAGACAATGGTTAACTTCGGGTGGACTTGGTGCTATGGGTTTTGGATTGCCTGCTGCGAT
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CDC_Corinne     GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
CDC_Luna        GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
CDC_Cory        GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
CDC_Frontier    GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
ICCX860047-9    GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
Myles           GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
CDC_Alma        GGGCGCTGCTGTTGCTAACCCTGATGCTGTTGTTGTTGATATCGATGGGGATGGTAGTTTTATGATGAATGTACAAGAGTTAGCTACTAT
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CDC_Corinne     AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
CDC_Luna        AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
CDC_Cory        AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
CDC_Frontier    AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
ICCX860047-9    AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
Myles           AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
CDC_Alma        AAAAGTGGAGAAACTCCCTGTTAAGATTTTGTTGTTGAATAATCAGCACTTGGGTATGGTTGTTCAGTGGGAGGATAGATTCTACAAGTC
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CDC_Corinne     GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
CDC_Luna        GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
CDC_Cory        GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
CDC_Frontier    GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
ICCX860047-9    GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
Myles           GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
CDC_Alma        GAATAGAGCTCATACTTATCTTGGTGACCCTTCTAGGGAGAATGAAATTTTCCCTAACATGCTTGGATTTGCAGATGCTTGTGGGATACC
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CDC_Corinne     AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
CDC_Luna        AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
CDC_Cory        AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
CDC_Frontier    AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
ICCX860047-9    AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
Myles           AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
CDC_Alma        AGCAGCTCGTGTGACGAAGAAGGAAGAGCTTAGAGATGCTATTCAGAAAATGTTGGATACTCCTGGTCCTTATCTTCTAGATGTTATTGT
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CDC_Corinne     ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGA------------------------------
CDC_Luna        ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTG
CDC_Cory        ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGG---------------
CDC_Frontier    ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTG
ICCX860047-9    ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTG
Myles           ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTG
CDC_Alma        ACCTCATCAAGAGCATGTTTTGCCAATGATTCCTAGTAATGGTTCCTTCAAGGATGTGATCACTGACGGTGATGGAAGAAGGAGTTACTG
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CDC_Corinne     --------------------------------------------------------------------------------------
CDC_Luna        ATTGATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTG
CDC_Cory        --------------------------------------------------------------------------------------
CDC_Frontier    ATTGATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTG
ICCX860047-9    ATTGATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTG
Myles           ATTGATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTG
CDC_Alma        ATTGATTGGGCTAAACTAGATACGGTATTCCTTCACTGTTGTTTTGTACAATATATATAGCTATTATTGCTATCCTAGTTGCGGGATTTG

CDC_Corinne     --------------------------------------------------------------------------------------
CDC_Luna        ACACTCGTTGTAAGCTAAGCATGT----------------------------------------------------------------
CDC_Cory        --------------------------------------------------------------------------------------
CDC_Frontier    ACACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTGTAATTTTGGTGGCATGTTTCTTTGTAGAATGCCGCACCTCTTGTTGTT
ICCX860047-9    ACACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTGTAATTTTGGTGGCATGTTTCTTTGTAGAATGCCGCACCTC--------
Myles           ACACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTGTAATTTTGGTGGCATGTTTCTTTGTAGAATGCCGCACCTCTTGTTGTT
CDC_Alma        ACACTCGTTGTAAGCTAAGCATGTTAGTTTAGTTTTTTGTTGTAATTTTGGTGGCATGTTTCTTTGTAGAATGCCGCACCTCTTGTTGTT

CDC_Corinne     -------------------------
CDC_Luna        -------------------------
CDC_Cory        -------------------------
CDC_Frontier    GTATTGTTTTTTTTTTCTTTT-----
ICCX860047-9    -------------------------
Myles           GTATTGTTTTTTTTTTCTTTTCTGTA
CDC_Alma        GTTTTTTTTT----------------
```

Appendix 5: AHAS1 amino acid multiple alignment *of Arabidopsis thaliana, Medicago spp, Cicer arietinum* and *Lens Culinaris*

Red amino acids = 5' or 3' UTR. Highlighed areas indicate common mutations sites known to cause Group 2 herbicide resistance

```
Arabidopsis thaliana            ----------------------------------LCFSISCSSFS-TMAAATTTTTTSSSISFSTKPSPSSSKSP
Medicago truncatula 'Caliph'    --------------------------QDKTTTLIHSCKLSHSAFTSTMAATTTTTPSRSPFHSHPPFP--KRTT
Medicago littoralis 'Angel'     ------------------LSLTKTILKQDKTTTLIHSCKLSHSAFTSTMAATTTTTPSRSPFYSHPPFP--KRTT
C.arietinum CDC Frontier genome ------------------------------------------------MAATTTTTPSRSAFSSSSHPIFPKSNT
C.arietinum IMI-S consensus     ------------------------------------------------MAATTTTTPSRSAFSSSSHPIFPKSNT
C.arietinum IMI-R consensus     ----------------------------------------------------------PSRSAFSSSSHPIFPKSNT
L.culinaris Redberry IMI-S      ------------------------------------------------------------------------------
L.culinaris Impact IMI-R        QESKERSKTTSVT-YDSTIL-TRQDT-IFILLHFKLKLYLHSTMAAAAATTTTTTSRSPFTSSSSSYSTFLKRNS

Arabidopsis_thaliana            LPISRFSLPFSLNPNKSSSSSRRRGIKSSSPSSISAVLNTTTNVTTTPSPTKPTKPETFISRFAPDQPRKGADIL
Medicago truncatula 'Caliph'    TLTFPLSPILN---KPKTTHSLI-GISCSLKPS-TSPPSTTTTVDEPFTS-----------RFSSTQPRKGSDIL
Medicago littoralis 'Angel'     TLTFPLSPILN---KPKTTHSLI-GISCSLKPS-TAPPSPTTTDDEPFTS-----------RFSSSQPRKGSDIL
C.arietinum CDC Frontier genome KLTFSLSPIFN---KPKLISSRPFKISSSLSKSPTAPSSITTTTTTTTTSTFI-------SRFSPTEPRKGSDIL
C.arietinum IMI-S consensus     KLTFSLSPIFN---KPKLISSRPFKISSSLSKSPTAPSSITTTTTTTTTSTFI-------SRFSPTEPRKGSDIL
C.arietinum IMI-R consensus     KLTFSLSPIFN---KPKLISSRPFKISSSLSKSPTAPSSITTTTTTTTTSTFI-------SRFSPTEPRKGSDIL
L.culinaris Redberry IMI-S      ------------------------------------------------------------------------------
L.culinaris Impact IMI-R        TLTLPFSPIYN---KPQSIHNRPLTVSSSLSNYPVAPASTTATTPDDQYI----------SRFSSTEPRKGADIL

Arabidopsis thaliana            VEALERQGVETVFAYPGGASMEIHQALTRSSSIRNVLPRHEQGGVFAAEGYARSSGKPGICIATSGPGATNLVSG
Medicago truncatula 'Caliph'    VEALEREGVTNVFAYPGGASMEIHQALTRSKTIRNILPRHEQGGVFAAEGYARSSGLPGVCIATSGPGATNLVSG
Medicago littoralis 'Angel'     VEALEREGVTNVFAYPGGASMEIHQALTRSKTIRNILPRHEQGGVFAAEGYARSSGLPGVCIATSGPGATNLVSG
C.arietinum CDC Frontier genome VEALEREGVTNVFAYPGGASMEIHQALTRSKIIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSG
C.arietinum IMI-S consensus     VEALEREGVTNVFAYPGGASMEIHQALTRSKIIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSG
C.arietinum IMI-R consensus     VEALEREGVTNVFAYPGGASMEIHQALTRSKIIRNVLPRHEQGGIFAAEGYARSSGLPGVCIATSGPGATNLVSG
L.culinaris Redberry IMI-S      ------------------------------------------------------------IATSGPGATNLVSG
L.culinaris Impact IMI-R        VEALERQGVTNVFAYPGGASMEIHQALTRSKTIRNILPRHEQGGVFAAEGYARSSGLPGVCIATSGPGATNLVSG
                                                                                              **************

Arabidopsis thaliana            LADALLDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVEDIPRIIEEAFFLATSGRPGPVLVD
Medicago truncatula 'Caliph'    LADALMDSVPLIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILDVEDIPRVVKEAFFLATSGRPGPVLID
Medicago littoralis 'Angel'     LADALMDSVPLIAITGQVLRRMIGTDAFQETPIVEVTRSITKHNYLILDVEDIPRVVKEAFFLATSGRPGPVLID
C.arietinum CDC Frontier genome LANALMDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILEVDDIPRVVREAFFVANSGRPGPVLID
C.arietinum IMI-S consensus     LANALMDSIPIIAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILEVDDIPRVVREAFFVANSGRPGPVLID
C.arietinum IMI-R consensus     LANALMDSIPIIAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILEVDDIPRVVREAFFVANSGRPGPVLID
L.culinaris Redberry IMI-S      LADALMDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLILDVDDIPRVVKEAFFLATSGRPGPVLID
L.culinaris Impact IMI-R        LADALMDSVPLVAITGQVPRRMIGTDVFQETPIVEVTRSITKHNYLILDVDDIPRVVKEAFFLATSGRPGPVLID
                                **:**:**:*::****** ******.*********************:::* ****   **** * ******** *
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76

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Arabidopsis thaliana            VPKDIQQQLAIPNWEQAMRLPGYMSRMPKPPEDSHLEQIVRLISESKKPVLYVGGGCLNSSDELGRFVELTGIPV
Medicago truncatula 'Caliph'    VPKDVQQQLAVPNWSEPIKLTGYLSRLPKIPGEAQLEQVLRLLLESEKPVLYVGGGCLNSSDELKRFVELTGVPV
Medicago littoralis 'Angel'     VPKDVQQQLAVPNWSEPIKLTGYLSRLPKIPGEAQLEQVLRLLLESKKPVLYVGGGCLNSSDELKRFVELTGVPV
C.arietinum CDC Frontier genome VPKDVQQQLAVPNWAQPIKLTGYLSRLPKIPIEAQLEQVVRLLLESKKPVLYVGGGCLNSSEELKRFVEITGIPV
C.arietinum IMI-S consensus    VPKDVQQQLAVPNWAQPIKLTGYLSRLPKIPIEAQLEQVVRLLLESKKPVLYVGGGCLNSSEELKRFVEITGIPV
C.arietinum IMI-R consensus    VPKDVQQQLAVPNWAQPIKLTGYLSRLPKIPIEAQLEQVVRLLLESKKPVLYVGGGCLNSSEELKRFVEITGIPV
L.culinaris Redberry IMI-S      VPKDIQQQLAVPNWAEPIKLTGYVSRLPKIPDESQFEQVVRLLLESKKPVLYVGGGCLNSSEELNRFVELTGIPV
L.culinaris Impact IMI-R        VPKDIQQQLAVPNWAEPIKLTGYVSRLPKIPDESQFEQVVRLLLESKKPVLYVGGGCLNSSEELNRFVELTGIPV
                                **** ***** ***     * ** ** ** *     **  **  ** ************** ** **** ** **

Arabidopsis_thaliana            ASTLMGLGSYPCDDELSLHMLGMHGTVYANYAVEHSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGK
Medicago truncatula 'Caliph'    ASTLMGLGSYPIGGEHSLSMLGMHGTVYANYAVDNSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGK
Medicago littoralis 'Angel'     ASTLMGLGSYPIGGEHSLSMLGMHGTVYANYAVDNSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGK
C.arietinum CDC Frontier genome ASTLMGLGSYPVGGEHSLQMLGMHGTVYANYAVDKSDLLLAFGVRFDDRVTGKLETFASRANIVHIDIDSAEIGK
C.arietinum IMI-S consensus     ASTLMGLGSYPVGGEHSLQMLGMHGTVYANYAVDKSDLLLAFGVRFDDRVTGKLETFASRANIVHIDIDSAEIGK
C.arietinum IMI-R consensus     ASTLMGLGSYPVGGEHSLQMLGMHGTVYANYAVDKSDLLLAFGVRFDDRVTGKLETFASRANIVHIDIDSAEIGK
L.culinaris Redberry IMI-S      ASTLMGLGSYPIGGEHSLHMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGK
L.culinaris Impact IMI-R        ASTLMGLGSYPIGGEHSLHMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGK
                                ***********   * ** **************  ******************** ***** *************

Arabidopsis_thaliana            NKTPHVSVCGDVKLALQGMNKVLENRAEELKLDFGVWRNELNVQKQKFPLSFKTFGEAIPPQYAIKVLDELTDGK
Medicago truncatula 'Caliph'    NKIPHLSICADMKVALEGLNRVLESKGIKGKLDFEAWRQELNVQKLKFPLGFKTFEDAISPQYAIQVLDELTNGD
Medicago littoralis 'Angel'     NKIPHLSICADMKVALEGLNRVLESKGIKGKLDFEAWRQELNVQKLKFPLGFKTFEDAISPQYAIQVLDELTNGD
C.arietinum CDC Frontier genome NKLPQVSVCADMKFALQGLNRILESKGIKDKLDFESWREELNVVKIKFPLGFKTFEDAISPQYAIQVLDELTNGD
C.arietinum IMI-S consensus     NKLPQVSVCADMKFALQGLNRILESKGIKDKLDFESWREELNVVKIKFPLGFKTFEDAISPQYAIQVLDELTNGD
C.arietinum IMI-R consensus     NKLPQVSVCADMKFALQGLNRILESKGIKDKLDFESWREELNVVKIKFPLGFKTFEDAISPQYAIQVLDELTNGD
L.culinaris Redberry IMI-S      NKIPHMSICADMKVALEGLNRVLESKGVKGKLDFEAWRQELNVQKLKFPLGFKTFENAISPQYAIQVLDELTNGD
L.culinaris Impact IMI-R        NKIPHMSICADMKVALEGLNRVLESKGVKGKLDFEAWRQELNVQKLKFPLGFKTFENAISPQYAIQVLDELTNGD
                                ** *  * * * * ** * *  **      ****  ** **** * **** ****  ** ***** ****** *

Arabidopsis thaliana            AIISTGVGQHQMWAAQFYNYKKPRQWLSSGGLGAMGFGLPAAIGASVANPDAIVVDIDGDGSFIMNVQELATIRV
Medicago truncatula 'Caliph'    AIVSTGVGQHQMWSAQFYKYKRPRQWLTSGGLGAMGFGLPAAIGAAVANPDAIVVDIDGDGSFMMNVQELATIRV
Medicago littoralis 'Angel'     AIVSTGVGQHQMWSAQFYKYKRPRQWLTSGGLGAMGFGLPAAIGAAVANPDAIVVDIDGDGSFMMNVQELATIRV
C.arietinum CDC Frontier genome AIVSTGVGQHQMWAAQFYKYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDAVVVDIDGDGSFMMNVQELATIKV
C.arietinum IMI-S consensus     AIVSTGVGQHQMWAAQFYKYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDAVVVDIDGDGSFMMNVQELATIKV
C.arietinum IMI-R consensus     AIVSTGVGQHQMWAAQFYKYKRPRQWLTSGGLGAMGFGLPAAMGAAVANPDAVVVDIDGDGSFMMNVQELATIKV
L.culinaris Redberry IMI-S      AIISTGVGQHQMWAAQFYEYKRPRQWLTSGGLGAMGFGLPAAIGAAVANPNAVVVDIDGDGSFIMNVQELATIRV
L.culinaris Impact IMI-R        AIISTGVGQHQMWAAQFYEYKRPRQWLTSGGLGAMGFGLPAAIGAAVANPNAVVVDIDGDGSFIMNVQELATIRV
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Arabidopsis thaliana           ENLPVKVLLLNNQHLGMVMQWEDRFYKANRAHTFLGDPAQEDEIFPNMLLFAAACGIPAARVTKKADLREAIQTM
Medicago truncatula 'Caliph'   ENLPVKILLLNNQHLGMVVQWEDRFYKANRAHTYLGDPSKEDEIFPNMLGFADACGIPAARVTKKEELREAIQKM
Medicago littoralis 'Angel'    ENLPVKILLLNNQHLGMVVQWEDRFYKANRAHTYLGDPSKEDEIFPNMLGFADACGIPAARVTKKEELREAIQKM
C.arietinum Frontier AHAS1      EKLPVKILLLNNQHLGMVVQWEDRFYKSNRAHTYLGDPSRENEIFPNMLGFADACGIPAARVTKKEELRDAIQKM
C.arietinum IMI-S consensus    EKLPVKILLLNNQHLGMVVQWEDRFYKSNRAHTYLGDPSRENEIFPNMLGFADACGIPAARVTKKEELRDAIQKM
C.arietinum IMI-R consensus    EKLPVKILLLNNQHLGMVVQWEDRFYKSNRAHTYLGDPSRENEIFPNMLGFADACGIPAARVTKKEELRDAIQKM
L.culinaris_Redberry IMI-S     ENLPIKILLLNNQHLGMVVQWEDRFYKSNRGHTYLGDPSREEEIFPNMLGFADACGIPAARVTKKEELREAIQKM
L.culinaris_Impact IMI-R       ENLPIKILLLNNQHLGMVVQWEDRFYKSNRGHTYLGDPSREEEIFPNMLGFADACGIPAARVTKKEELREAIQKM
                               * ** * *********** ******** ** ** ****  * ******* ** ************  ** *** *

Arabidopsis_thaliana           LDTPGPYLLDVICPHQEHVLPMIPSGGTFNDVITEGDGRIKY-EMKPVIIRTFYGLCMHMVKKLSLQFPVCFGNL
Medicago truncatula 'Caliph'   LETPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITEGDGRRSY-LLEQNEI-YIP--------------------
Medicago littoralis 'Angel'    LETPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITEGDGRRSY-LLEQNEI-YIPYVLFCTIYKIIVIIVA-F-D-
C.arietinum_Frontier_AHAS1     LDTPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITDGDGRRSY-LIGLN-IRYSFTVVLYNIYSYYCYPSCGI-H-
C.arietinum IMI-S consensus    LDTPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITDGDGRRSY-LIGLN-IRYSFTVVLYNIYSYYCYPSCGI-H-
C.arietinum IMI-R consensus    LDTPGPYLLDVIVPHQEHVLPMIPSNGSFKDVITDGDGRRSY-LIGLN-IRYSFTVVLYNIYSYYCYPSCGI-H-
L.culinaris_Redberry IMI-S     LDTPGPYLLDVITPHQEHVLPMIPSNGSFKDVITEGDGRTSY-FLGPK-DMIFLHVCFVQ-I-LMLS-L-DLMA-
L.culinaris_Impact IMI-R       LDTPGPYLLDVITPHQEHVLPMIPSNGSFKDVITEGDGRTSY-FLGPK-DMIFLHVCFVQ-I-LMLS-L-DLMA-
                               * ********** ************ * * **** ****  *
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Appendix 6: IMI Herbicide ratings and KASP SNP genotyping results

Table 6.1. Comparison of RILI MI treatment phenotypic response to results of the KASP allele specific genotyping assay

Plant Sample	Herbicide Trial 1	Herbicide Trial 2	Marker	Does marker accurately predict Phenotype?
CDC 512-51 Parent DNA Sample 1	A	A	A	YES
CDC 512-51-2 Parent DNA Sample 2	A	A	A	YES
ICCx860047-1 Parent DNA Sample 1	B	B	B	YES
ICCx860047-2 Parent DNA Sample 2	B	B	B	YES
512-51/ICCX860047-9 1	B	B	B	YES
512-51/ICCX860047-9 2	A	A	A	YES
512-51/ICCX860047-9 3	B	B	B	YES
512-51/ICCX860047-9 5	A	A	A	YES
512-51/ICCX860047-9 6	A	A	A	YES
512-51/ICCX860047-9 7	A	A	A	YES
512-51/ICCX860047-9 8	A	A	A	YES
512-51/ICCX860047-9 9	B	B	B	YES
512-51/ICCX860047-9 10	A	A	A	YES
512-51/ICCX860047-9 12	A	A	A	YES
512-51/ICCX860047-9 13	B	B	B	YES
512-51/ICCX860047-9 14	A	A	X	MISSING DATA
512-51/ICCX860047-9 16	B	X	B	YES
512-51/ICCX860047-9 18	B	B	B	YES
512-51/ICCX860047-9 19	B	B	B	YES
512-51/ICCX860047-9 20	X	B	B	YES
512-51/ICCX860047-9 21	B	X	B	YES
512-51/ICCX860047-9 22	B	B	B	YES
512-51/ICCX860047-9 23	X	A	A	YES
512-51/ICCX860047-9 24	B	B	B	YES
512-51/ICCX860047-9 25	A	A	A	YES
512-51/ICCX860047-9 26	A	A	A	YES
512-51/ICCX860047-9 27	B	B	B	YES
512-51/ICCX860047-9 28	B	B	B	YES
512-51/ICCX860047-9 29	A	A	A	YES
512-51/ICCX860047-9 34	A	A	X	MISSING DATA
512-51/ICCX860047-9 35	B	B	B	YES
512-51/ICCX860047-9 36		X	A	MISSING DATA
512-51/ICCX860047-9 37	B	B	B	YES

512-51/ICCX860047-9 38	A	A	A	YES
512-51/ICCX860047-9 39	A	A	A	YES
512-51/ICCX860047-9 41	B	X	B	YES
512-51/ICCX860047-9 42	X	X	X	
512-51/ICCX860047-9 43	B	B	B	YES
512-51/ICCX860047-9 44	A	X	A	YES
512-51/ICCX860047-9 45	X	A	X	MISSING DATA
512-51/ICCX860047-9 46	B	B	B	YES
512-51/ICCX860047-9 49	A	A	A	YES
512-51/ICCX860047-9 52	A	X	A	YES
512-51/ICCX860047-9 53	B	X	B	YES
512-51/ICCX860047-954	B	B	B	YES
512-51/ICCX860047-9 56	A	A	A	YES
512-51/ICCX860047-9 57	A	A	A	YES
512-51/ICCX860047-9 58	A	A	A	YES
512-51/ICCX860047-9 60	X	B	B	YES
512-51/ICCX860047-9 62	A	A	X	MISSING DATA
512-51/ICCX860047-9 64	A	A	A	YES
512-51/ICCX860047-9 65	A	A	A	YES
512-51/ICCX860047-9 66	A	A	A	YES
512-51/ICCX860047-9 68	X	A	A	YES
512-51/ICCX860047-9 69	X	A	X	MISSING DATA
512-51/ICCX860047-9 70	A	A	A	YES
512-51/ICCX860047-9 71	A	A	A	YES
512-51/ICCX860047-9 72	X	A	A	YES
512-51/ICCX860047-9 74	B	B	B	YES
512-51/ICCX860047-9 75	X	B	B	YES
512-51/ICCX860047-9 76	X	B	B	YES
512-51/ICCX860047-9 79	B	B	B	YES
512-51/ICCX860047-9 80	B	B	B	YES
512-51/ICCX860047-9 81	B	B	B	YES
512-51/ICCX860047-983	A	A	A	YES
512-51/ICCX860047-9 86	A	A	B	NO
512-51/ICCX860047-9 87	X	A	A	YES
512-51/ICCX860047-9 88	B	B	B	YES
512-51/ICCX860047-9 89	A	A	A	YES
512-51/ICCX860047-9 90	X	A	A	YES
512-51/ICCX860047-9 91	A	A	A	YES
512-51/ICCX860047-9 92	A	X	A	YES
512-51/ICCX860047-9 93	X	A	--	MISSING DATA
512-51/ICCX860047-9 94	B	X	B	YES
512-51/ICCX860047-9 98	B	X	B	YES
512-51/ICCX860047-9 101	A	X	A	YES

Appendix 7: Chromosome Genetic Maps

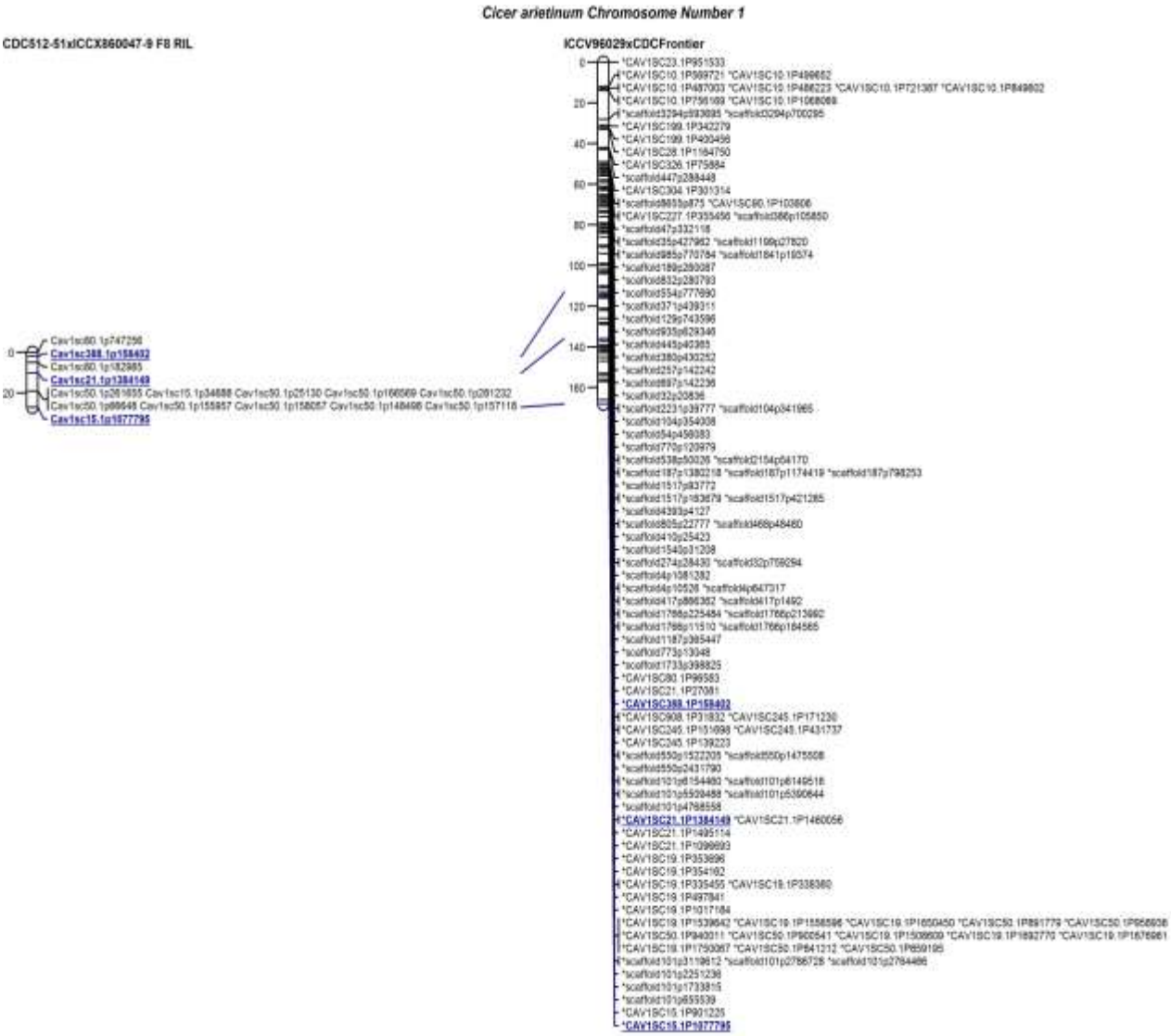

Figure 5.1. *Cicer arietinum* Chromosome Number 1

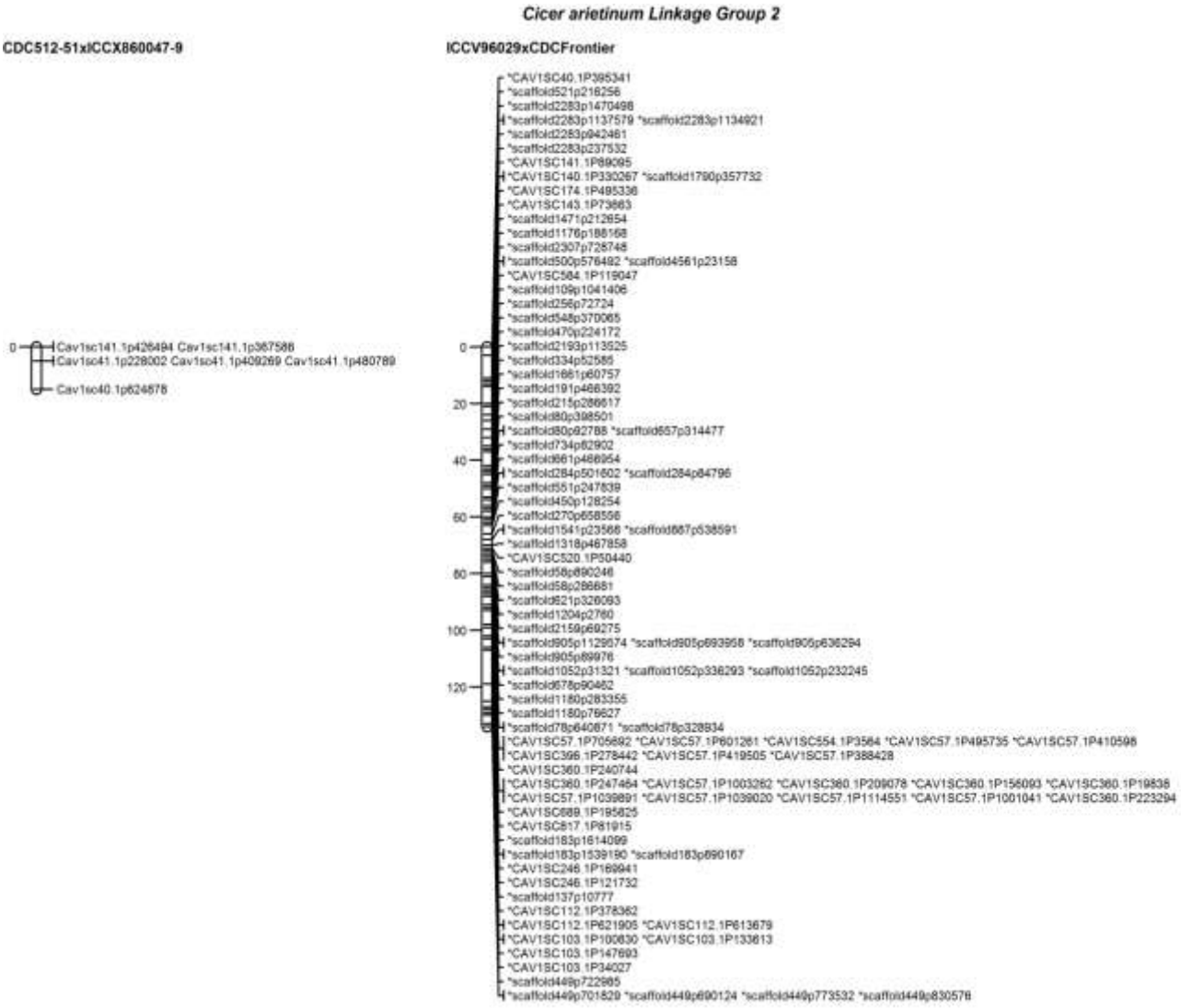

Figure 5.2. Cicer arietinum Linkage Group 2

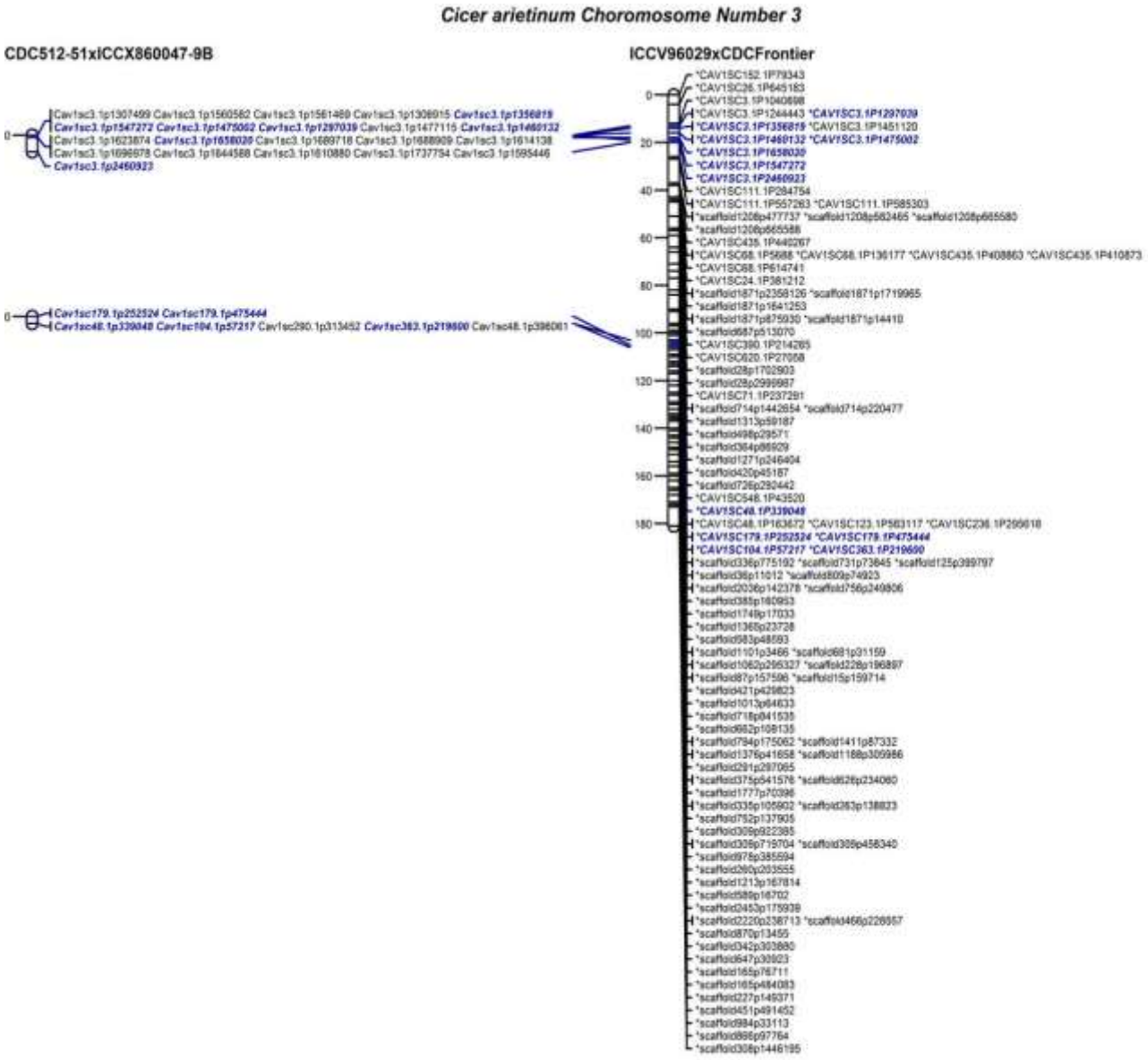

Figure 5.3. *Cicer arietinum* Chromosome Number 3

Figure 5.4 *Cicer arietinum* Chromosome Number 4

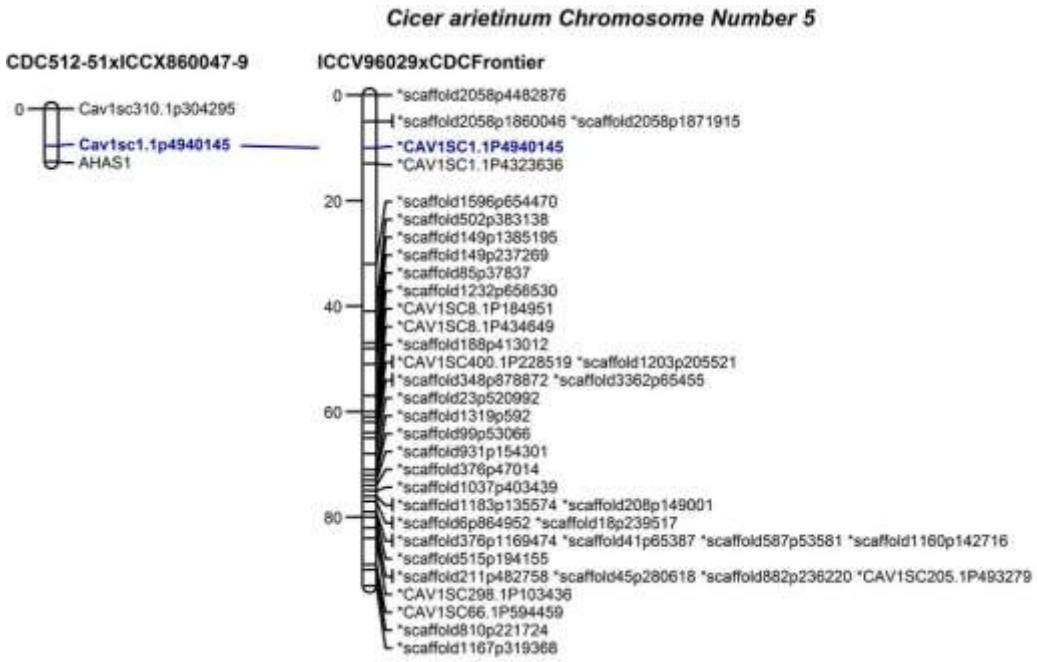

Figure 5.5. *Cicer arietinum* Chromosome Number 5

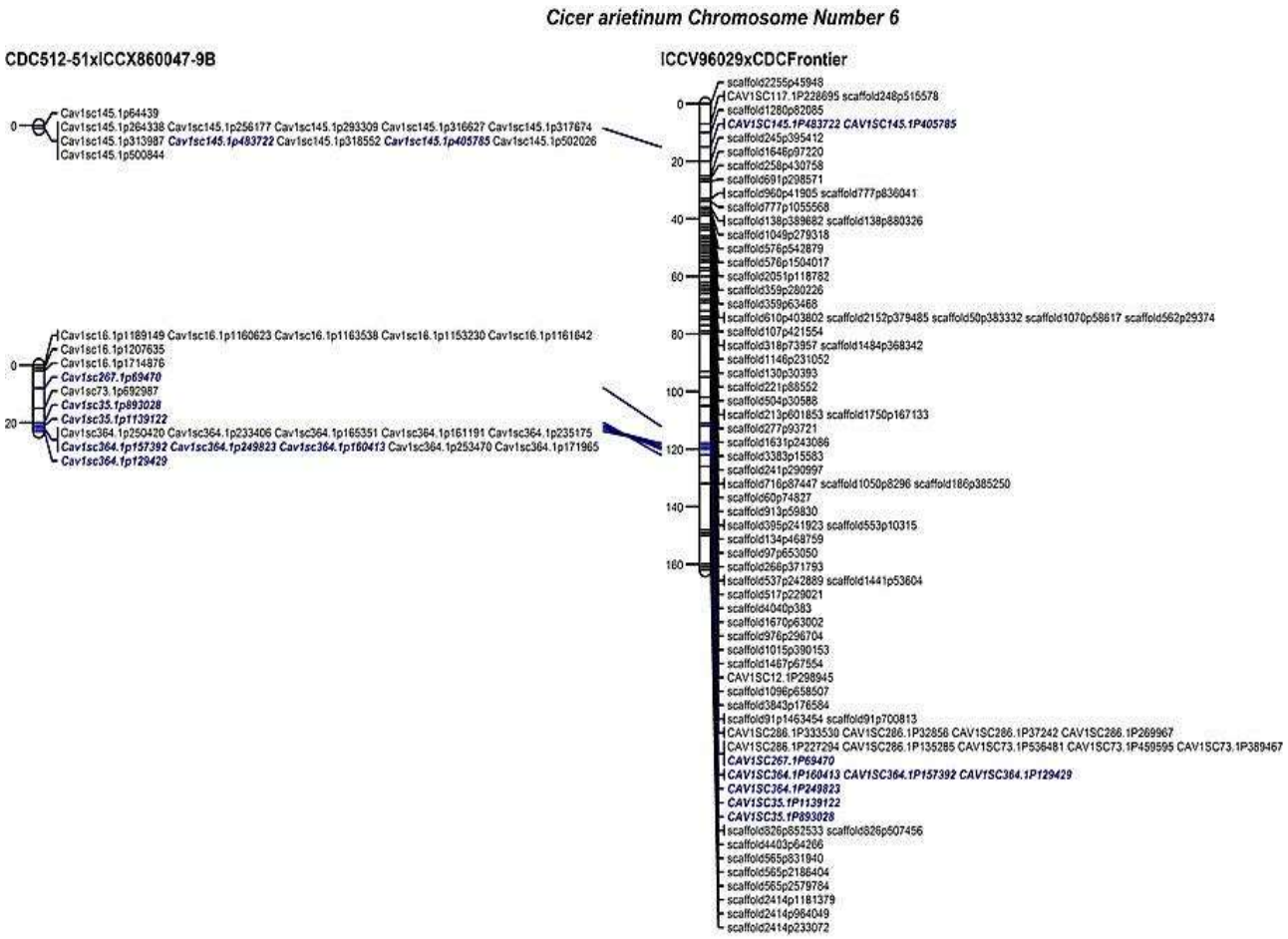

Figure 5.6 *Cicer arietinum* Chromosome Number 6

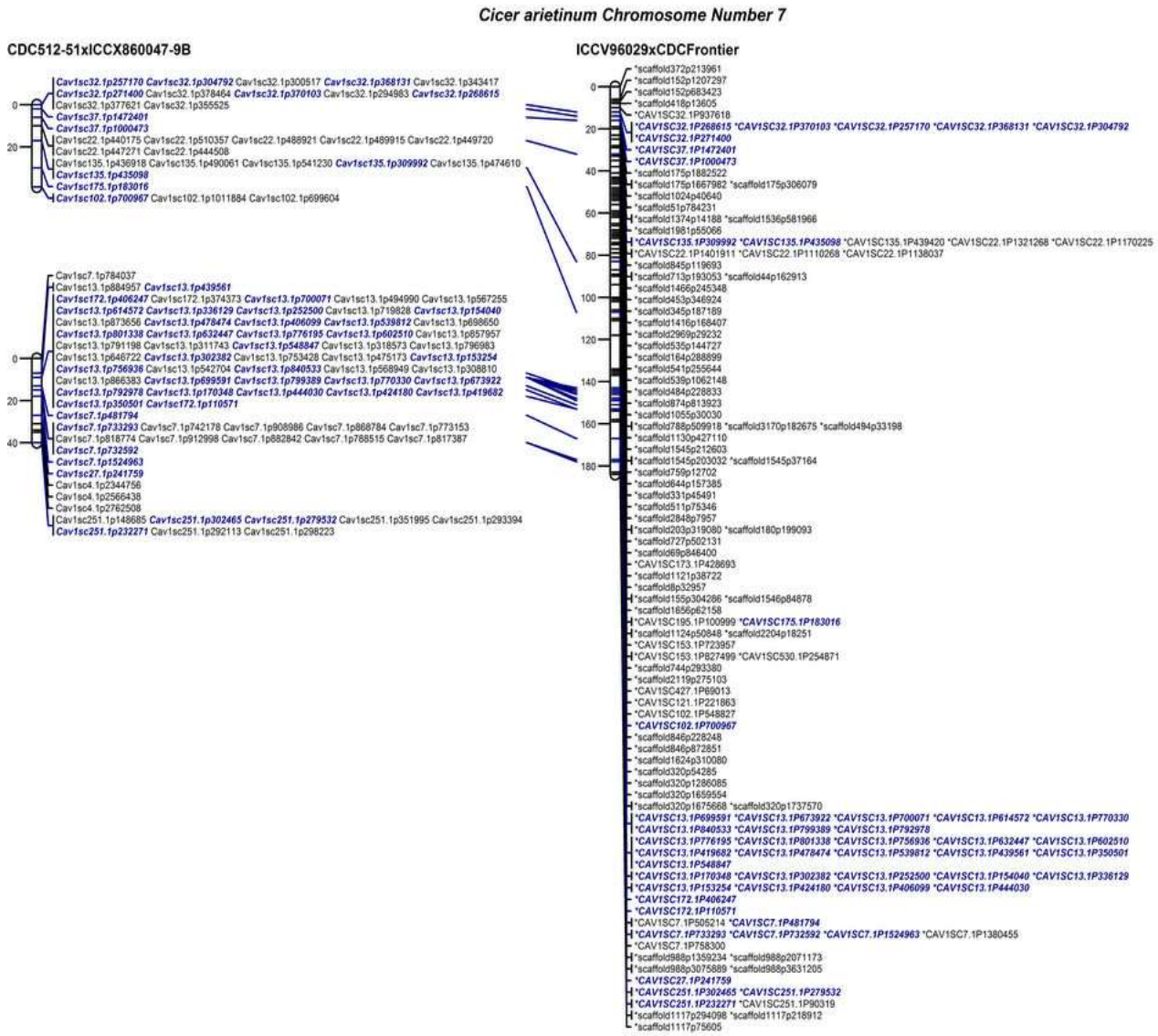

Figure 5.7. *Cicer arietinum* Chromosome Number 7

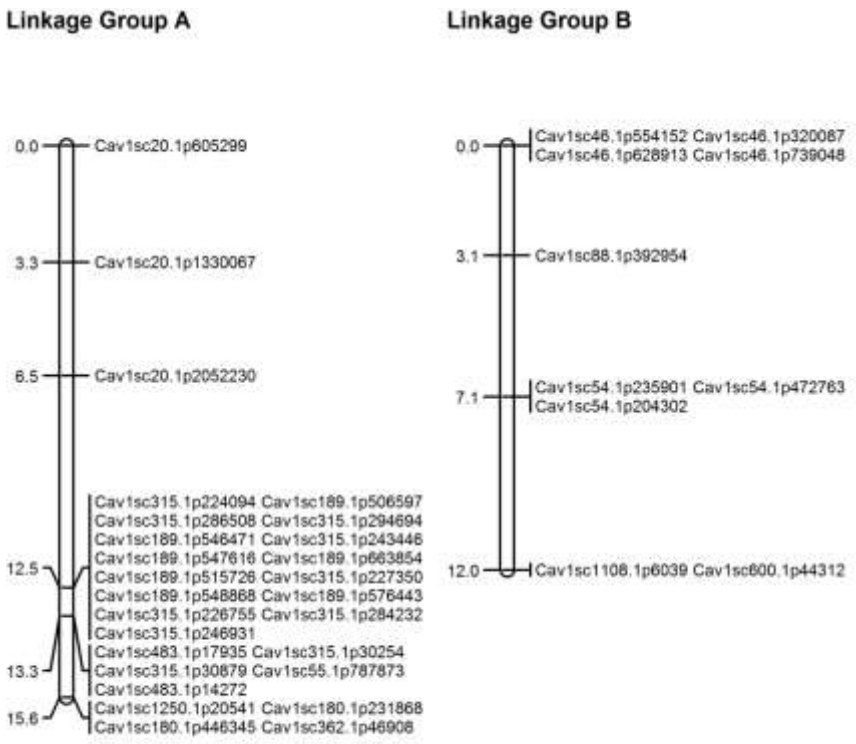

Figure 5.8. Other linkage groups not linked to reference chickpea map

Appendix 7: AHAS Enzyme Activity Additional Data

Table 6.1. AHAS activity of resistant and susceptible genotypes in complex with IMI herbicide using AHAS specific colorimetric assay.

Chickpea Variety	*In vivo* colorimetric assay			*In vitro* colorimetric assay		
	Time after IMI treatment	Mean Absorbance (520nm, 3rep)	Acetolactate Concentration (µM)	IMI concentration (µM)	Mean Absorbance (520nm, 2rep)	Acetolactate Concentration (µM)
CDC Frontier	0 hr	1.015	282.74	0	0.81	221.92
	2 hr	1.679	476.51	3	1.26	353.91
	4 hr	1.467	414.58	6	1.15	323.13
	6 hr	0.240	56.68	8	0.09	12.20
	12 hr	0.195	43.65	10	0.06	3.74
	24 hr	0.077	9.23	12	0.00	0.00
	5 days	-0.096	0.0	14	0.00	0.00
CDC Cory	0 hr	0.608	163.92	0	0.42	108.61
	2 hr	0.770	211.18	3	0.43	112.10
	4 hr	0.771	211.57	6	0.42	109.48
	6 hr	0.730	199.61	8	0.31	76.95
	12 hr	0.687	187.16	10	0.35	89.35
	24 hr	0.638	172.87	12	0.36	93.00
	5day	0.652	176.86	14	0.35	87.60

Table 6.2. ANOVA results for in vitro colorimetric assay comparing the factors IMI concentration, genotype and their interaction affecting the response variable acetolactate concentration (Microsoft Excel output)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
IMI concentration	184295.9	6	30715.99	606.1524	4.36E-16	2.847726
Genotype	8116.646	1	8116.646	160.1747	4.71E-09	4.60011
Interaction	136597.5	6	22766.26	449.2715	3.51E-15	2.847726
Within	709.4319	14	50.67371			
Total	329719.6	27				

Table 6.3 ANOVA for *in vivo* colorimetric assay comparing two genotypes for the acetoin production from 0 to 120 hours after IMI treatment

Time Interval	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
0hr	Between Groups	21174.72	1	21174.72	35.73732	0.003935	7.708647
	Within Groups	2370.04	4	592.51			
	Total	23544.76	5				
2hr	Between Groups	105604.50	1	105604.50	429.1484	3.21E-05	7.708647
	Within Groups	984.32	4	246.08			
	Total	106588.82	5				
4hr	Between Groups	61821.05	1	61821.05	235.4582	0.000105	7.708647
	Within Groups	1050.23	4	262.56			
	Total	62871.27	5				
6hr	Between Groups	30641.50	1	30641.50	20.08925	0.010972	7.708647
	Within Groups	6101.07	4	1525.27			
	Total	36742.57	5				
12hr	Between Groups	30696.70	1	30696.69	28.27667	0.006015	7.708647
	Within Groups	4342.34	4	1085.58			
	Total	35039.03	5				
24hr	Between Groups	39799.04	1	39799.04	143.81517	0.00027712	7.708647
	Within Groups	1106.95	4	276.74			
	Total	40905.99	5				
120hr	Between Groups	46916.58	1	46916.58	53.996754	0.0018265	7.708647
	Within Groups	3475.51	4	868.88			
	Total	50392.09	5				