

Weed Management Update

Ramon G. Leon

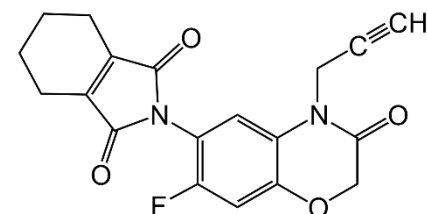
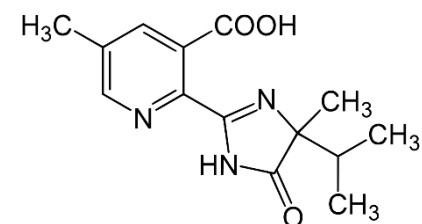
Summary

- Herbicide carryover risk
- Herbicide tolerance
- ALS-resistant lines
- Weed risk assessment

Experimental design

Treatments

- Two herbicides:
 - Imazapic (Cadre®), flumioxaxine (Valor®)
- Six application times (Herbicide carryover):
 - 24, 18, 12, 6, 3 and 0 months before planting
- Five doses (Herbicide as preemergent):
 - 1X, 0.5X, 0.25X, 0.125X and 0.068X
- Control without herbicides
- Completely randomized block design
 - 3 x 3 m plot as experimental unit
 - 4 reps each treatment



Soil sampling and greenhouse bioassay

Soil sampling

- Undisturbed soil cores
 - 60 cm depth
 - Two cores per experimental plot
 - Bioassay and residue analysis



Greenhouse bioassay

- Cores placed in a special bench
 - 2 mm depth furrow
 - Carinata seed planted each 2 cm



Soil sampling and greenhouse bioassay

Herbicide residues analysis

- Soil cores
 - Split in 5 cm increments
 - Homogenized
- High performance liquid chromatography–mass spectrometry
 - Agilent-6120 Infinity; Agilent Technologies, Inc., Wilmington, DE
- A rapid resolution high-definition column
 - Agilent ZORBAX RRHD SB-C18; Agilent Technologies, Inc., Wilmington, DE



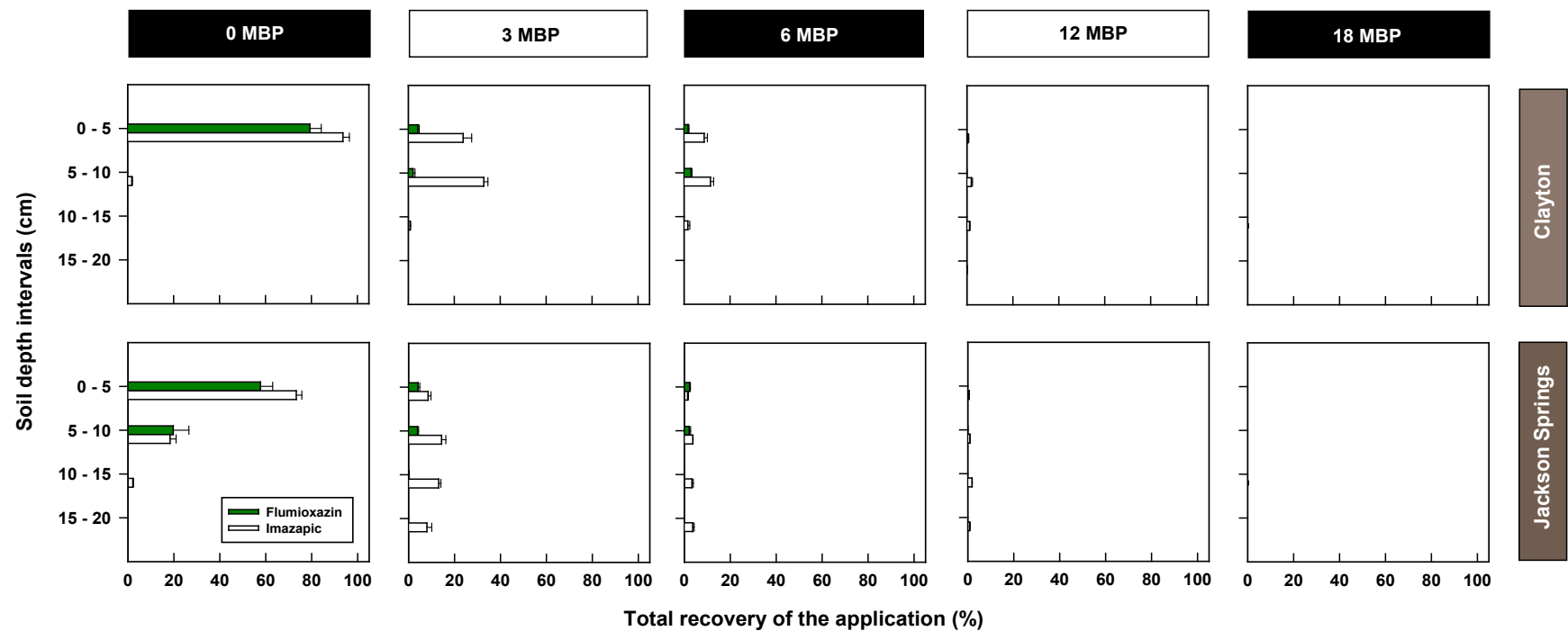


Figure 1 Effect of the herbicide application interval before carinata planting on total recovery of two herbicides in soil from two locations of North Carolina. Imazapic and flumioxazin were applied using recommended label rates of 70 and 107 g ai ha⁻¹, respectively. MBP: Months before planting. Soil sampling depths were increments of 5 cm from 0 to 20 cm. Error bars represent the standard error of the mean (n=3).

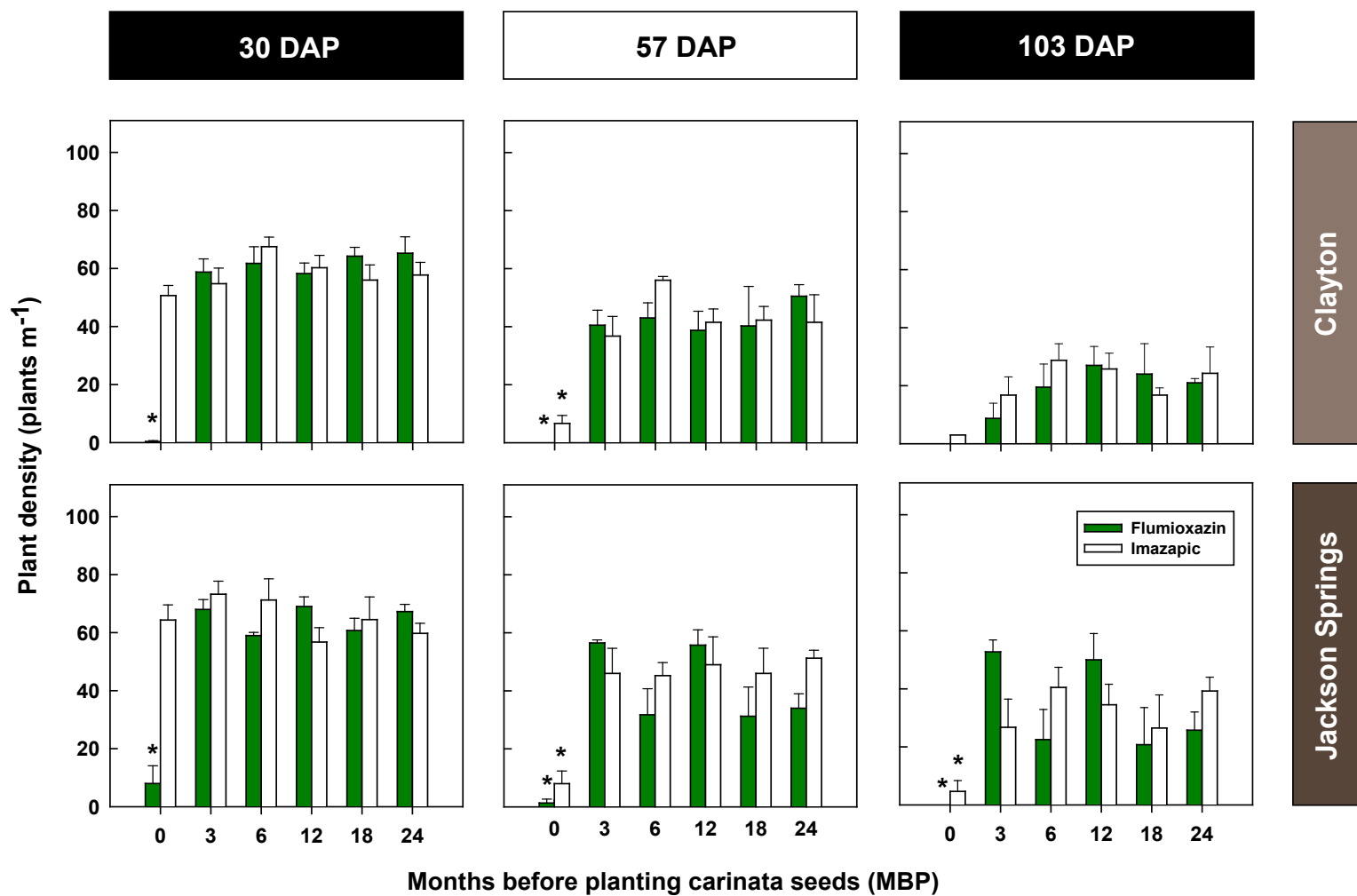


Figure 2 Carinata population density in response to application interval before planting for two herbicides in *B. carinata* in two locations of North Carolina. The evaluations were done 30, 57, and 103 days after carinata planting (DAP). Error bars represent the standard error of the mean (n=4). * Indicates significant differences with the control without herbicide, according with Dunnett-Test (p-value < 0.05).

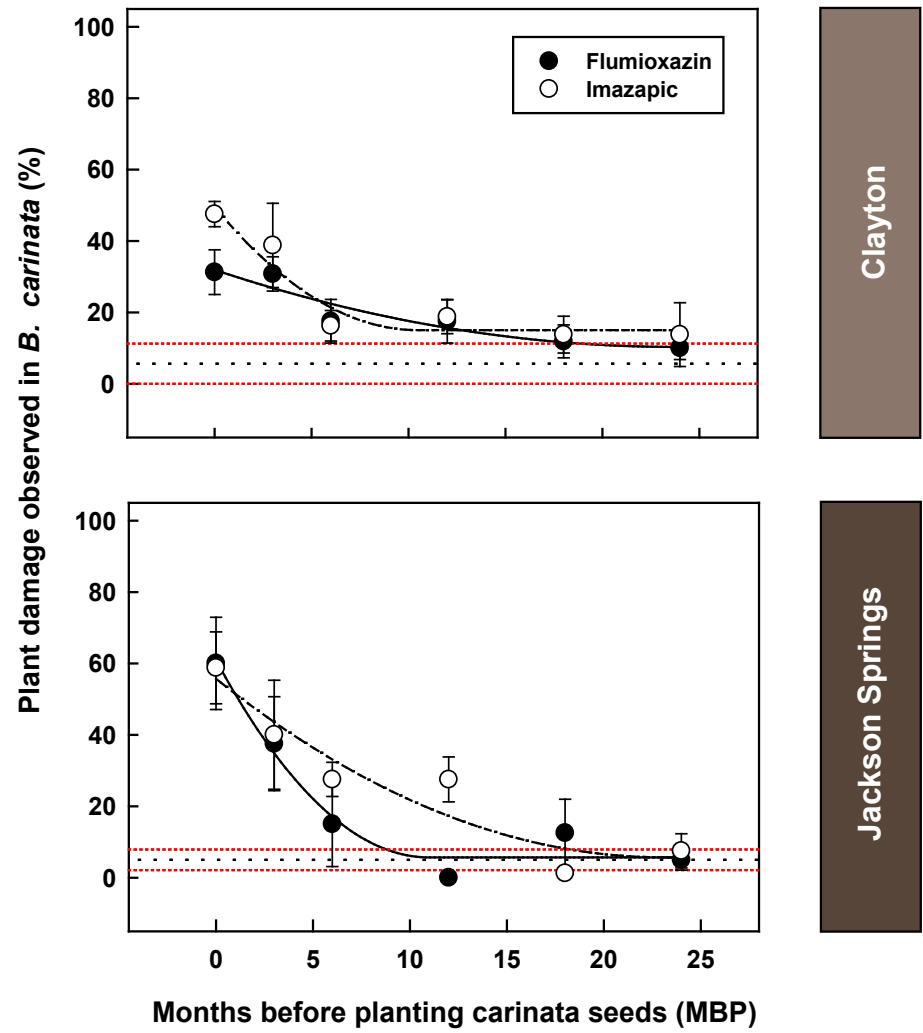


Figure 3 Plant damage at different soil depths in soil cores collected from two locations of North Carolina in response to application interval before carinata planting for two herbicides in *B. carinata*. Black solid and discontinuous lines represent the best-fit model for imazapic and flumioxazin, respectively. Error bars represent the standard error of the mean (n=4). Continuous red lines indicate the average plant damage observed in the control treatment and discontinuous red lines represent standard error.

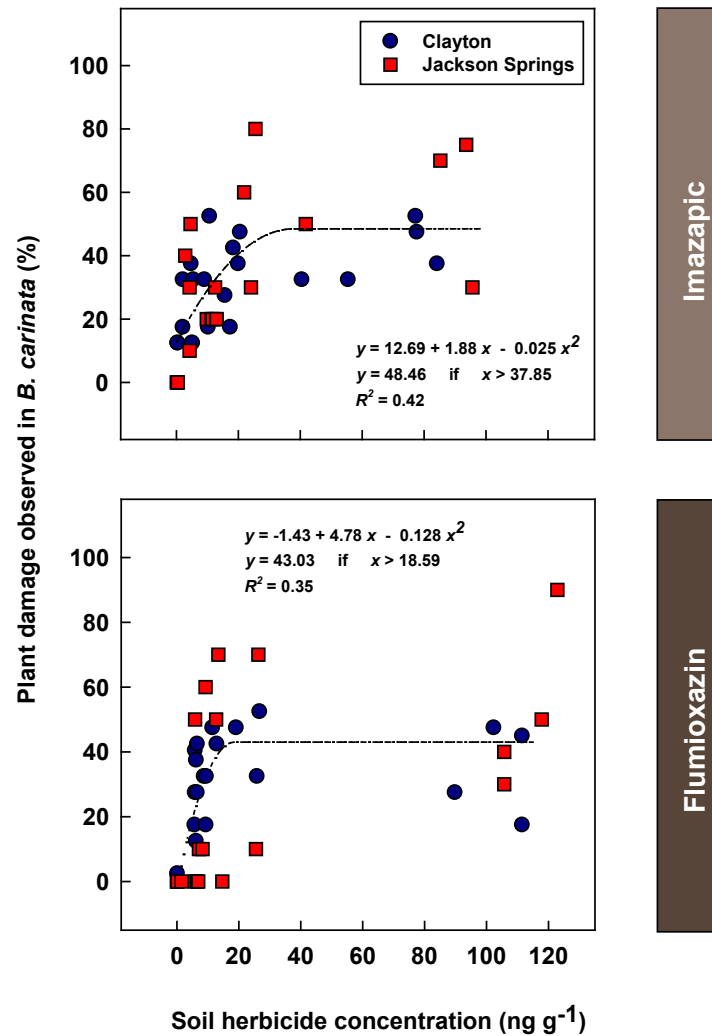


Figure 6 Soil herbicide recovered amount (expressed as concentration) and its effect on carinata plant damage observed in two locations of North Carolina. Black discontinuous lines represent the best-fit model selected for each herbicide.

Acetolactate synthase (ALS) Resistant Lines

➤ Acetohydroxyacid synthase



➤ Absent in animals



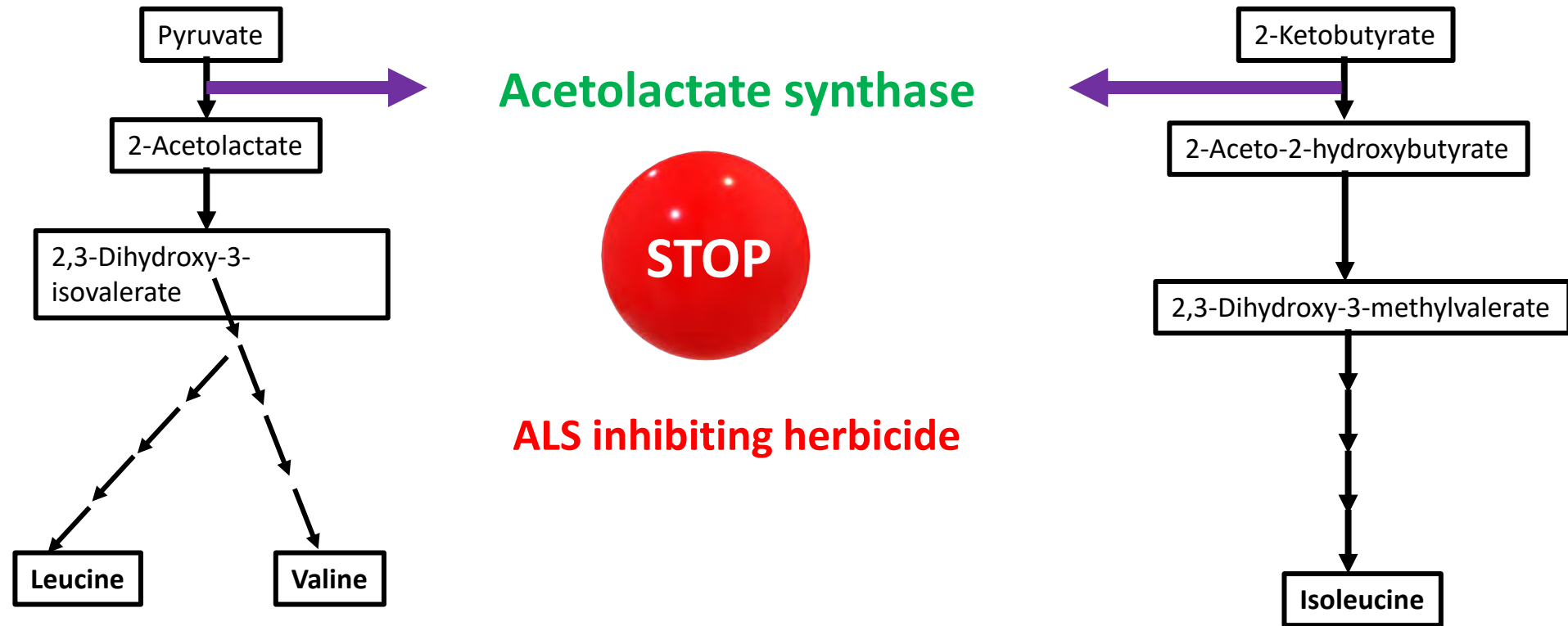
➤ Found in plants and microorganisms

➤ Condensation of two pyruvate molecules to form acetolactate

➤ First step in the biosynthesis of branched-chain amino acids

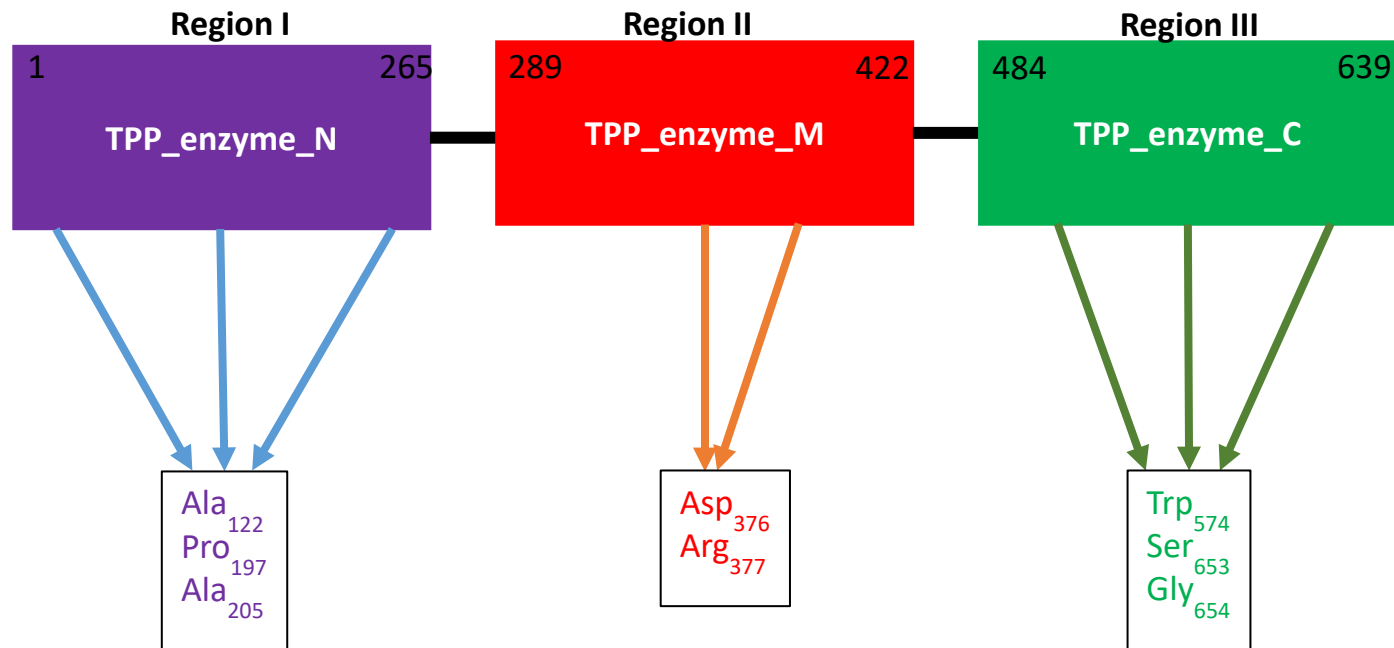
➤ Important for biosynthesis of amino acid Valine, Leucine and Isoleucine

ALS inhibition



Mutation reported

Over the past 27 years, 29 resistance-endowing amino acid substitutions at eight positions of the ALS gene in 66 weed species have been identified



In *Brassica carinata* ALS gene was found in two copies one was present in ChrB05 and the another was present on ChrB09

<http://www.weedscience.org/mutations/MutationDisplayAll.aspx>

Singh et al. Pest Manag Sci. 2019;75:1131-1139


Region I multiple sequence alignment ChrB05

Canola2	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
Canola1	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS012.0032	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS012.0672	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
AVANZA641	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
AVANZA642	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ChrB05	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
CL19B4.2962	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS012.0081	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS012.0082	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
CL19B4.2951	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
CL19B4.2952	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
CL19B4.2961	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS012.0031	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
ALS007.0671	1	MEIHQALTRSSSTIRNVLPRHEQGGVFAAEGYARSSGKPGIC IATSGPGATNLVSGLADAM
Canola2	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
Canola1	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS012.0032	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS012.0672	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
AVANZA641	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
AVANZA642	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ChrB05	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
CL19B4.2962	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS012.0081	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS012.0082	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
CL19B4.2951	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
CL19B4.2952	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
CL19B4.2961	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS012.0031	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT
ALS007.0671	61	LDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNYLVMDVDDIPRIVQEAFFLAT

No mutation found

Region I multiple sequence alignment ChrB09

Threonine to Isoleucine (Not reported)

		70	80	90	100	110	120
ALS007.0671	TE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
CL19B4.2961-R	TE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
CL19B4.2962-R	TE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ALS012.0031-R	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ALS012.0032-R	TE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ALS012.0081-R	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ALS007.0672	TE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
CL19B4.2952-R	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ALS012.0082-R	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
CANOLA2	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
CANOLA1	TD	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
ChrC09:26880203-26882370	ID	KN	KTFV	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
AVANZA641	IE	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			
AVANZA642	ID	KN	KTFI	ISRYAPDEPRKGADILVEALERQGVETVFAYPGGASMEIHQALTRSSTIRNVL			

One new mutation Iso to Thr was found

Region II multiple sequence alignment

	190	200	210	220	230	240
ChrC09:26880203-26882370rev	TAGCAGGGCTAA	ATTGTGCACAT	GACATTGATTCT	GCTGAGATTGGGAAGAA	T	AAGAC
ChrB05:13552239-13554406	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CL19B4.2952	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CL19B4.2961	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CL19B4.2962	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS012.0081	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS012.0082	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS012.0032	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CL19B4.2951	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS012.0031	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS007.0671	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
ALS007.0672	GAGCAGGGCTAA	GATTGTGCACAT	TGACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
AVANZA642	TAGCAGGGCTAA	ATTGTGCACAT	GACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
AVANZA641	TAGCAGGGCTAA	ATTGTGCACAT	GACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CANOLA1	TAGCAGGGCTAA	ATTGTGCACAT	GACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC
CANOLA2	TAGCAGGGCTAA	ATTGTGCACAT	GACATTGATTCT	GCTGAGATTGGGAAGAA	C	AAGAC

	250	260	270	280	290	300
ChrC09:26880203-26882370rev	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ChrB05:13552239-13554406	GCCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CL19B4.2952	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CL19B4.2961	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CL19B4.2962	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS012.0081	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS012.0082	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS012.0032	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CL19B4.2951	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS012.0031	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS007.0671	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
ALS007.0672	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
AVANZA642	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
AVANZA641	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CANOLA1	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			
CANOLA2	ACCTCAT	GTGTCTGTGTGTGGTGATGT	TAAGCTGGCTTTGCAAGGGATGAACAAGGTTCT			

No mutation found

Region III multiple sequence alignment ChrB05

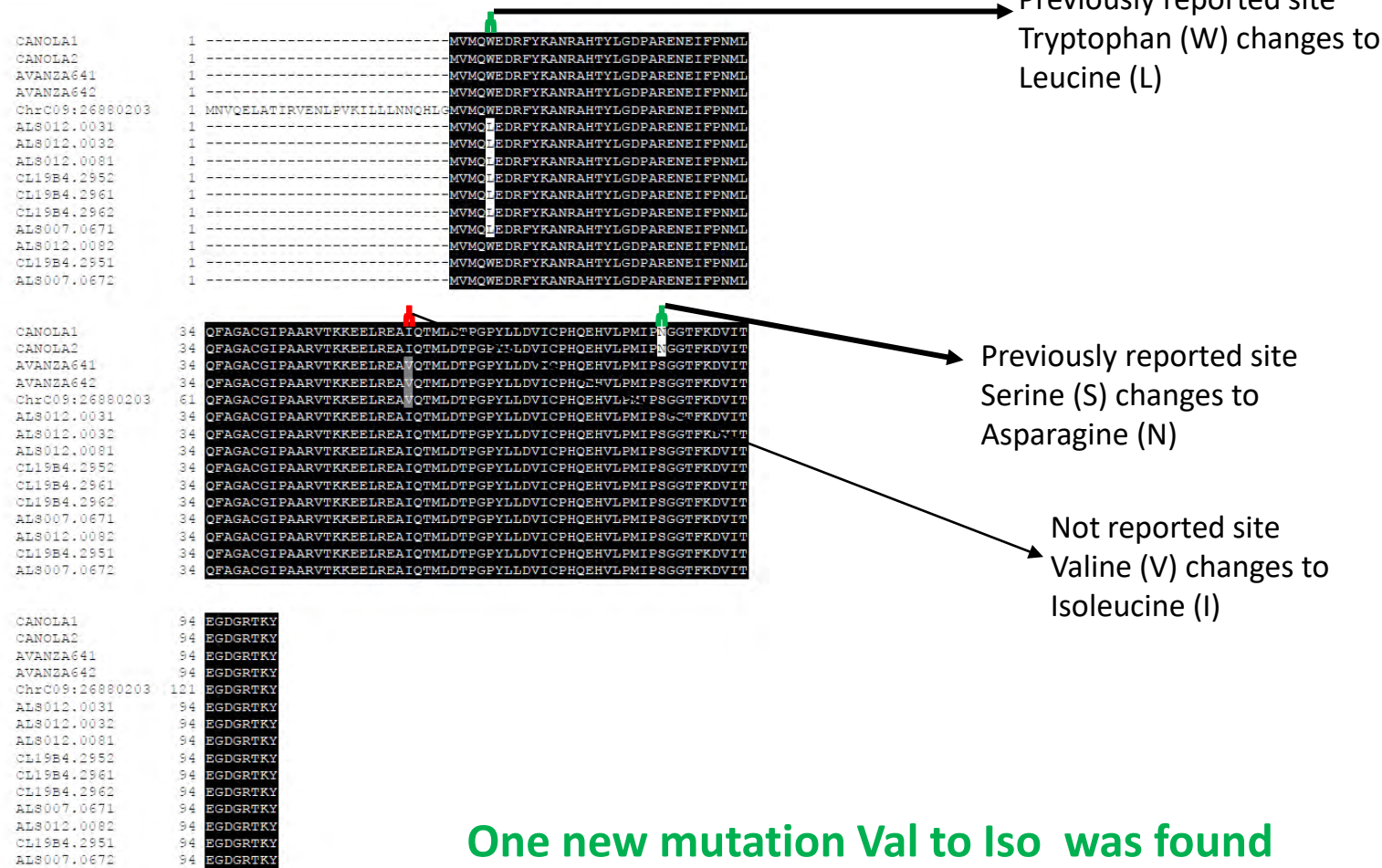
CANOLA1	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AL8012.0032	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
ChrB05:13552239	1	MSFGLPAAIGASVANFDAIVVDIDGDSGFFMNVOELATIRVENLPVKVLLNNQHLGMVM
CL19B4.2951	1	MSGGLPAAIGASVANFDAIVVDIDGDSGFFMNVOELATIRVENLPVKVLLNNQHLGMVM
AL8012.0031	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AL8012.0081	1	MS-LDSCCIGASVANFDAIVVDIDGDSGFFMNVOELATIRVENLPVKVLLNNQHLGMVM
AL8012.0082	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
CL19B4.2952	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
CL19B4.2961	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
CL19B4.2962	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AL8007.0671	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AL8007.0672	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AVANZA641	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
AVANZA642	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM
CANOLA2	1	-----MNVOELATIRVENLPVKVLLNNQHLGMVM

CANOLA1	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8012.0032	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
ChrB05:13552239	61	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
CL19B4.2951	61	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8012.0031	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8012.0081	60	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8012.0082	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
CL19B4.2952	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
CL19B4.2961	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
CL19B4.2962	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8007.0671	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AL8007.0672	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AVANZA641	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
AVANZA642	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP
CANOLA2	31	QWEDRFYKANRAHTYLGDPAKENEIPFNMLQFAGACGIPAARVTKKEELRDAIQTMLDTP

CANOLA1	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8012.0032	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
ChrB05:13552239	121	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
CL19B4.2951	121	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8012.0031	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8012.0081	120	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8012.0082	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
CL19B4.2952	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
CL19B4.2961	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
CL19B4.2962	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8007.0671	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AL8007.0672	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AVANZA641	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
AVANZA642	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY
CANOLA2	91	GPYLLDVICPHQEHVLPMPISGGTFKDVITEGDRTRY

No mutation found

Region III multiple sequence alignment ChrB09



Weed Risk Assessment

Assessment	Minimum score for rejection	Obtained Score	Outcome
Australian WRA			
Total	6	6	Needs further evaluation
Agricultural	--	2	--
Environmental	--	3	--
Pre Tool	11	6	Accept

Acknowledgements

